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Habitat and Associated Fauna of Four Species of Fish in Ontario Streams^{1, 2}

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ABSTRACT

Four species of fish found in Ontario streams can be divided into two distinct groups on the basis of habitat and associated fauna. *Salvelinus fontinalis* (Mitchill) and *Cottus bairdi* Girard are found associated in cool source waters whereas *Ambloplites rupestris* (Rafinesque) and *Micropterus dolomieu* Lacépède occur together in warmer downstream waters. The latter group has more associated species of fish than the former. Some invertebrates such as certain stonefly and mayfly nymphs occur frequently with fish of one group and rarely with fish of the other group. They may be useful as indicators of waters likely to be inhabited by or suitable for the species of fish with which they are frequently associated.

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INTRODUCTION

SOURCES OF DATA

EACH SUMMER since 1946 the Department of Planning and Development of the Province of Ontario has surveyed rivers in southern Ontario. Data from these surveys, on which the author worked in the summers of 1950, 1951, and 1952, were made available for this study. Data for Wilmot Creek, similar to that for the above surveys, were collected under the auspices of National Research Council of Canada.

DESCRIPTION OF WATERSHEDS. All the rivers and streams examined are in southern Ontario and drain into the Great Lakes except for the South Nation River which is a tributary of the Ottawa River.

The Ganaraska, Humber, Don, Wilmot, Mimico and Etobicoke are small watersheds bounded on the north by either the Niagara cuesta or moraines of the interlobate series or, in the case of the Humber, by both. The Humber River thus derives water from both sources. The Ganaraska and Don Rivers and Wilmot Creek originate in the interlobate moraines. Mimico Creek and Etobicoke Creek

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do not tap these main source areas but arise as drainage of the till plain. All these rivers and creeks enter Lake Ontario at intervals which extend over about 80 miles of the north shore in the Toronto region.

The Nith and adjacent Thames watersheds are similar to one another in some respects. Although part of the source of the Thames River is in till plains and moraines and that of the Nith River in clay plains, the upper ends of both tend to dry up in summer. Many of their upper waters are now drainage ditches and the land drained is agricultural and relatively low in contour.

The Speed and Saugeen Rivers flow southwestward on the inclined limestone plain to the west of the Niagara cuesta. That portion of the Saugeen surveyed originates from sources in this limestone and in the gravel moraines and kames of the horseshoe series in the vicinity. It flows mainly in the spillways of the antecedent glacial streams. The Speed, which drains the Guelph drumlin fields, also flows in old spillways. A large proportion of these two watersheds is forested.

The Ausable River flows westward into the southern end of Lake Huron. Most of its small headwater streams are now drainage ditches and the river is characterized by heavy spring floods and low summer flow.

The Moira and Napanee river flow into the northeastern end of Lake Ontario. Both have their headwaters in the region of Precambrian rocks of the Canadian Shield and their lower course and southern tributaries of Paleozoic limestone.

The South Nation River is the only one surveyed which does not drain into the Great Lakes. Its highest source area is located on a limestone plain northeast of the eastern end of Lake Ontario and from this it flows northeastward across a flat plain to the Ottawa River. Much of the South Nation dries up completely or to standing pools in late summer of dry years. Its gradient of only 2.5 feet per mile on the average, contrasts with that of the Saugeen, 9.5 feet per mile, and of the Speed, 20.0 feet per mile.

The Holland River flows north into Lake Simcoe. It is notable for its sandy source areas and its meandering downstream section in the Holland Marsh.

STREAM SURVEYS. Since most of the data presented in this study were selected from data collected on surveys by the Department of Planning and Development a short discussion of the purpose and methods of the surveys will be given.

The purpose of the surveys, as stated in the reports of the Department, was "to make a preliminary examination of the waters of the drainage basins and to classify them as to their present suitability for fish and secondly to make recommendations for possible improvements."

Prior to each summer's field work, places were selected in the watershed to be visited by the field crew. These "stations", located usually where a road crossed the river or stream, were as close as one half mile on small tributaries and as far apart as three or four miles on large rivers. Stations were also located above and below settlements on streams.

Information relating to conditions of the stream, both of the water and terrain, along with biological data, was recorded for each station.

Aquatic organisms were sampled as follows: fish were generally caught by one person using a six-foot seine although in large rivers other methods such as

gill netting, trap netting and angling were employed. Invertebrates were collected in a hand sieve or by picking with forceps from aquatic vegetation, stones, wood and other submerged objects. Habitats in still, rapid and evenly flowing water were sampled and invertebrates from each habitat were usually placed in separate vials.

Collections were later examined and the organisms classified and used to zone various sections of the river. Because of special requirements of their life cycles some aquatic insects indicate stream conditions not only at the time of the survey but also for other seasons not covered which may include the warmest weeks of summer. Potentialities of a stream for particular species of fish can thus be estimated. The collections of fish generally substantiated these findings at their particular stations.

THE PROBLEM

Ricker (1934) drew up an ecological classification of Ontario streams as an introduction to the study of Ontario waters. Ide (1935) and Sprules (1947) have shown that many aquatic insects are restricted in their distribution by the temperature of the water which is dependent in many instances on distance from the source and by rate of flow which depends mainly on stream gradient. Whitney (1939) found that the resistance of mayfly nymphs to high temperatures was correlated with the temperature of their respective habitats. Dymond (1939, 1947) and Radforth (1944) have noted that many fish are restricted in their distribution by temperature, and Fry (1951) and Hart (1952) have shown from laboratory experiments that selected and lethal temperatures of fish generally were correlated with the temperature of the habitat. However, little quantitative work has been done to determine the degree of association between aquatic invertebrates and fish that are similarly restricted by temperature and rate of flow and to justify the use of fish and aquatic insects or other invertebrates as indicators of conditions suitable for certain species of fish.

The many stream survey report forms of the Department of Planning and Development from 1946 to 1952 contain much data on physical conditions of streams, the fish found therein and the aquatic invertebrates associated with them. An analysis of the data was undertaken to ascertain how restricted certain species of fish are in their distribution and to determine what degree of association exists between these species and the organisms, other fish and invertebrates, found with them.

The problem resolved itself into showing that some species of fish are restricted in their distribution, that those restricted by similar requirements form groups which live in sections of streams of different average physical conditions and that certain other fish and aquatic invertebrates are associated with them in these regions. Some of these organisms are frequently associated with species of only one group and can therefore be used as indicators of waters likely to be inhabited by or suitable for the species of fish of that group.

METHODS OF ANALYSIS

CARDINAL SPECIES OF FISH

More than 60 species of fish were collected in the surveys but many were taken from only one watershed and others were of such ubiquitous distribution that

a detailed study of them was considered unprofitable for this study. Because of its widespread occurrence in cool lotic waters, it was decided to centre the study around the important game fish *Salvelinus fontinalis* (Mitchill) the eastern speckled trout. In addition to selecting for study stations at which this species was taken, consideration was given to the feasibility of analysing a random sample of all stations where *S. fontinalis* was not taken and thus of obtaining a picture of non-speckled trout waters. However, since absence of a species from a collection is not a guarantee of its absence from a locality it was decided to adopt a positive approach by using the presence of other species of fish and analyse data for these species for comparison and contrast with data for *S. fontinalis*.

Cottus bairdi Girard, the northern muddler, is often found associated with the eastern speckled trout and it was selected as the main species for comparison with the latter.

Members of the sunfish family Centrarchidae are generally found in warmer waters than the above species and were selected as the main species for contrast with the eastern speckled trout. Data for two species only, the rock bass, *Ambloplites rupestris* (Rafinesque), and the smallmouth bass, *Micropterus dolomieu* Lacépède, were used for final analysis since data for others of the sunfish family were meagre and mostly confined to one watershed.

The above four species of fish form the basis of this study and are hereafter referred to as the *cardinal* species. Their occurrence in the different watersheds is given in Table I.

TABLE I. Watersheds surveyed and number of stations at which the cardinal species were collected.

Watershed or part thereof	Year surveyed	In drainage basin of	Number of collection stations	Speckled trout	Northern muddler	Rock bass	Smallmouth bass
South Nation R.	1946, 1947	Ottawa River	0	1	16	1	
Napanee R.	1950	Lake Ontario	0	0	9	2	
Moira River	1947, 1948	" "	3	1	23	14	
Ganaraska R.	1947	" "	20	3	0	0	
Wilmot Creek	1947	" "	11	0	1	0	
Don River	1949	" "	2	3	0	0	
Humber River	1946	" "	22	14	14	0	
Mimico Creek	1949	" "	0	0	3	0	
Etobicoke Creek	1946,	" "	0	1	3	0	
Nith River	1949	Lake Erie	21	13	57	46	
Speed River	1952	" "	46	42	3	4	
Thames River	1946, 1950	Lake St. Clair	12	9	83	44	
Ausable River	1947	Lake Huron	1	0	7	0	
Saugeen River	1951	" "	99	72	3	0	
Holland River	1952	" "	6	17	0	1	
All rivers	1946-1952	St. Lawrence R.	243	176	222	112	

TREATMENT OF DATA AND SOURCES OF ERROR

For each of the four cardinal species, data from all survey forms on which its occurrence was recorded were tabulated. For example, for *S. fontinalis* there were 243 survey report forms representing an occurrence at 243 different stations of this cardinal species.

Averages were obtained for physical conditions of the habitat such as water and air temperatures, volume of flow, and percentage shade.

The total number of times each associated organism occurred with each cardinal species was determined. These figures were then converted to percentage occurrences since the size of the sample naturally differed for each cardinal species.

Most sources of error are related to the field work although several possible errors in analysis of data and interpretation of findings will be discussed.

One source of error is in the variation in collecting methods employed over the years. For instance, in some early surveys collections of both fish and invertebrates were made at all dried-to-pools stations whereas not all such stations were sampled in later surveys. Sampling, therefore, of fish and invertebrates living in still and perhaps quite warm waters was not consistent in all surveys. Sometimes at these stations only fish or invertebrates were collected; since data were used only from stations at which both types of organisms were taken a further inconsistency is introduced.

Another source of error is the bias introduced by collecting some groups of invertebrates more intensely in some surveys than in others. In the survey years 1946 to 1950 most kinds of invertebrates were collected although the orders Ephemeroptera and Plecoptera were emphasized. In 1951 and 1952 this emphasis was increased and members of these two orders made up the bulk of the invertebrate collections. These were the years of the surveys of the Saugeen and Speed rivers when most of the trout and muddler were taken; it follows, therefore, that the occurrence of invertebrates other than Ephemeroptera and Plecoptera is less with these species of fish than it would have been if the sampling had been uniform. At static water stations there is a limited fauna of mayflies and stoneflies so that in all surveys there was probably a more comprehensive collection of other invertebrates at such stations.

Data used to assess the degree of association between fish and associated organisms are qualitative in the sense that kinds of organisms rather than frequency or abundance at one location are considered but quantitative in the sense that number of kinds and number of locations where each kind was taken are used. Because only the presence of an organism at a station or, in the case of invertebrates, in one type of water (rapid, evenly flowing, still) has been used, abundant forms receive the same weight as rare forms providing the latter were collected.

In some surveys the rapid-water, evenly-flowing and still-water categories for invertebrates were grouped and some vials contained organisms from both rapid and evenly-flowing water, or occasionally from both evenly-flowing and still water, in addition to the three separate categories listed above. This made separation into two divisions difficult, so that figures for some of the invertebrates to be presented later, representing occurrences in rapid and still water, are only approximate.

Speed of current where each species of fish was collected was not recorded. At many stations both rapid and quiet water were present. With a cardinal species from rapid water such as *S. fontinalis*, all species collected at the same station whether taken from rapid or still water were used to determine which species of fish could be considered to be associates of *S. fontinalis*.

Relatively the same amount of collecting effort was attempted at each station but owing to variation in size of streams and the number of different habitats involved, it was difficult to achieve uniform effort. Personal differences in collecting effort and errors in identifications of collections were inevitable as more than 12 different persons were engaged in the surveys.

Accurate identification was facilitated through the use of reference collections. The names of the fish of the reference collection and of some individual specimens were checked by Dr W. B. S. Scott of the Royal Ontario Museum of Zoology. The reference collection of invertebrates was checked by Dr F. P. Ide of the University of Toronto, who verified also the names of the species of *Ephemeroptera*.

Another inconsistency in the data is introduced by the fact that most stations were visited only once during a survey. Stations of a watershed such as the Saugeen might have been visited in May when the water was still cool or in late summer when the water was quite warm. In 1952, one whole watershed, the Holland, was examined in the late summer when it was warm, whereas the Speed watershed was surveyed in May and June. In north temperate regions, some invertebrates such as certain mayflies are present as nymphs in late spring in waters which in July and August are too warm for all stages except the diapausing eggs. For instance, Ide (1935) has shown that "the emergence at the lower stations of the species represented also at the upper stations is confined more and more to the early part of the season as we go downstream." He concludes that for *Iron pleuralis* Banks at a downstream station, "the eggs remain in an unhatched condition for at least four months," i.e., from the beginning of June to early October. Because of this some invertebrates which occur frequently in cool trout waters and have a high percentage occurrence with the speckled trout are sometimes found in May and early June in warmer waters and, therefore, may show an infrequent occurrence with the smallmouth bass.

Sometimes permanent cold source waters occur in warmer downstream sections of streams. These source waters may be cold springs in the bottom of the stream or cold tributaries; both would provide limited habitat for cold water species. The presence of these local cold source waters in some warm downstream sections of streams may account for some of the apparent discrepancies in which indicators of cool waters suitable for *S. fontinalis* show a small percentage occurrence with *A. rupestris* and *M. dolomieu*.

PHYSICAL CONDITIONS OF HABITATS

Analysis of data of physical conditions of the habitats of the four cardinal species of fish was made only for those conditions which were consistently noted at the collection stations and which lend themselves to quantitative treatment. These are distance from source, temperature, shade, cattle pollution, percentage of still water and volume of flow.

DISTANCE FROM SOURCE

For each station where a cardinal species occurred—more than 500 stations in all—measurements were made on topographic maps to determine the distance of that section of the stream from the source. For convenience, categories of

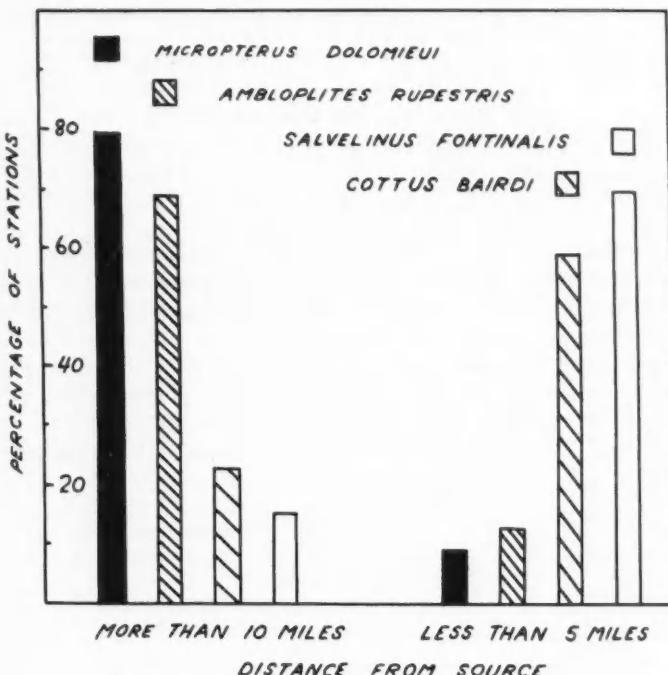


FIG. 1. Percentage of headwater and downstream stations where each cardinal species was collected.

distance in miles from the source were chosen as follows: less than 1, 1 to 2, 2 to 3, 3 to 5, 5 to 10, 10 to 20, 20 to 30, etc.

For each cardinal species the percentage of stations at which it was collected that were more than 10 and less than 5 miles from the source is given in Fig. 1. By this arbitrary division of most of the distance categories listed above into two categories, more than two-thirds of all stations for each cardinal species are included and clearly show that the smallmouth bass and rock bass are typically downstream species and the muddler and speckled trout occur most frequently in headwaters.

TEMPERATURE

At each station simultaneous measurements of air and water temperature were usually made only once. Sometimes this was in the coolness of an early morning in spring or in the heat of a late afternoon in summer. Because of such differences in time of day and season of observations, average water temperatures for cardinal species are not strictly comparable. However, Table II shows that the average air temperatures of the habitat of the cardinal species are very similar, indicating that differences in time and season of observations were about the same

TABLE. II. Average temperatures at the sampling stations for cardinal species of fish.

Cardinal species	Average temperatures at stations	
	Air °F	Water °F
<i>Micropterus dolomieu</i> , smallmouth bass	73.4	70.6
<i>Ambloplites rupestris</i> , rock bass	72.1	69.3
<i>Cottus bairdi</i> , northern muddler	73.2	61.9
<i>Salvelinus fontinalis</i> , speckled trout	73.4	60.3

for each cardinal species so that the average water temperatures can, with discretion, be used in habitat comparison. The habitats are compared in Fig. 2 on the basis of water temperature and distance from source, the latter being one of the main factors controlling the former.

Temperature of a stream depends also on the extent of overhanging banks and amount of vegetation growing along the banks. Both shade the stream from the sun. Amount of shade provided by vegetation depends on direction of stream and time of day. At each station an estimate was made of the percentage of the

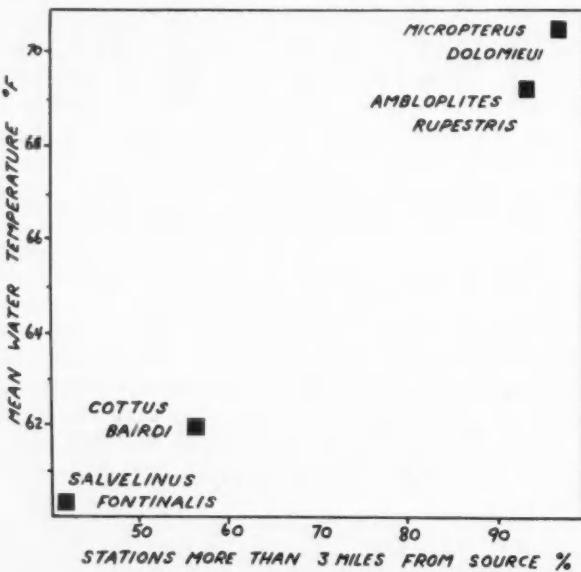


FIG. 2. Illustration of the grouping of cardinal species into two dissimilar habitats as defined by temperature and distance from source.

stream in shade. Average percentage of shade has been plotted against average water temperature in Fig. 3 which shows that the centrarchids occurred in warm waters with little shade whereas the speckled trout and muddler were taken in cool shaded waters.

CATTLE POLLUTION

Although heavy pollution by cattle was noted as such, usually only the fact that cattle obviously had access to the stream was recorded. Seventy-eight per cent of the stations where the rock bass and smallmouth bass were taken showed evidence of cattle pollution. For the muddler and speckled trout the percentage of stations with such evidence was 45 and 43, respectively. The high percentage for the centrarchids reflects the predominantly agricultural character of the areas drained by the Nith and Thames rivers which contributed more than half the records of occurrence of the two species. Much lower percentages for the trout and muddler were not unexpected since most occurrences of these species were recorded in the Saugeen and Speed rivers, much of which is located in glacial spillways and other wooded areas not suitable for agriculture.

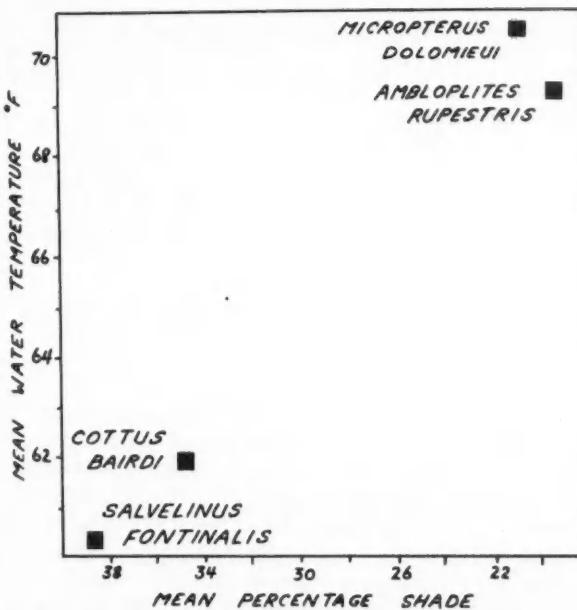


FIG. 3. Habitats of the cardinal species compared from two related aspects, temperature and shade.

RATE AND VOLUME OF FLOW

In the lower reaches of streams the volume of water is often large and gradient slight so that percentage of still water is, in part, related to volume of flow. In Fig. 4 the percentage of stations at which each cardinal species was taken having a discharge greater than 10 cubic feet per second and the average percentage of still water of all stations where each species was collected are used to illustrate

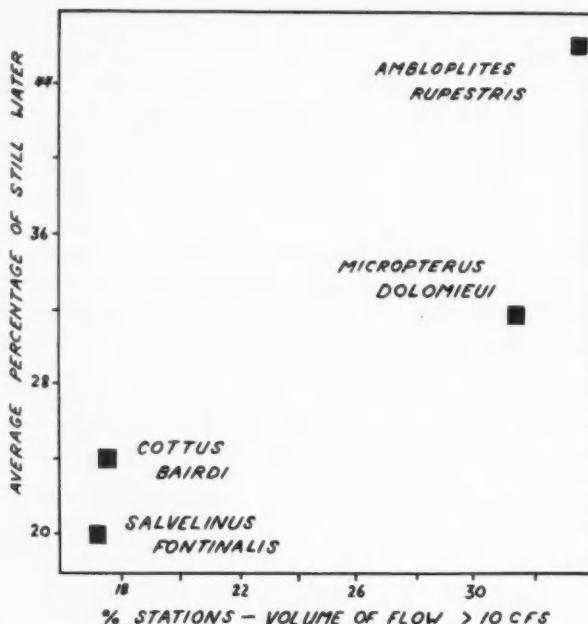


FIG. 4. Comparison of habitats of the cardinal species on the basis of proportion of still water and volume of flow.

that the speckled trout and muddler occur in smaller streams with more rapids than do the centrarchids.

ASSOCIATED FAUNA

Associated with the four cardinal species of fish are other fish and invertebrates. Only the presence and not the relative abundance of an associated organism with a cardinal species has been used in the analysis of data. With few exceptions, the scientific names of fishes are those used in the 1957 edition of "The Fishes of Ohio" by Milton B. Trautman. Four species listed do not occur in Ohio waters. Their names follow the nomenclature used in the 1947 edition (1952 printing) of "The Fishes of the Great Lakes Region" by C. L. Hubbs and K. F. Lagler. The northern muddler is *Cottus bairdi bairdi* Girard as described in the latter publication. However, only two fishes in this paper have been given subspecific names.

Table III lists the cardinal species, the sample size of each (the number of stations at which each was collected), and the number of species of fish found with them. A grouping of the cardinal species is again evident. More species were found associated with the centrarchids than with the speckled trout and muddler. The sample sizes of the cardinal species are such that few if any additional associated species would be added if the samples were larger. A greater variety

TABLE III. Number of species of fish found with each cardinal species.

Cardinal species	No. of stations	No. of associated species of fish
<i>A. rupestris</i> , rockbass	222	58
<i>M. dolomieu</i> , smallmouth bass	112	49
<i>S. fontinalis</i> , speckled trout	243	39
<i>C. bairdi</i> , northern muddler	176	33

of species of fish is found with the cardinal species of downstream waters. This agrees with Shurrager's findings in the Hocking River of Ohio (Shurrager, 1932) where he found 52 species in large streams, 32 in small streams and 18 species in headwaters.

TABLE IV. Association of cardinal species with each other. Figures beneath a top heading represent the percentage occurrence of that species with the species shown at the side. For example, *M. dolomieu* occurred at 34% of all locations where *A. rupestris* was collected, but *A. rupestris* occurred in 68% of all collections in which *M. dolomieu* was taken. All figures are to the nearest whole percentage.

Cardinal species	No. of stations	Percentage occurrence of associated species			
		<i>Micropterus dolomieu</i>	<i>Ambloplites rupestris</i>	<i>Cottus bairdi</i>	<i>Salvelinus fontinalis</i>
<i>Micropterus dolomieu</i>	(112)	100	68	0	1
<i>Ambloplites rupestris</i>	(222)	34	100	1	0
<i>Cottus bairdi</i>	(176)	0	0	100	57
<i>Salvelinus fontinalis</i>	(243)	1	0	42	100

Table IV gives the percentage occurrence of smallmouth bass, rock bass, muddler, and the eastern speckled trout with each other, and shows that the smallmouth bass is commonly associated with the rock bass and that the speckled trout and the muddler are associates.

Seven of the species found more often with the centrarchids than with the speckled trout and muddler are given in Table V. Each of these seven species,

TABLE V. Seven species of fish associated more often with *M. dolomieu* and *A. rupestris* than with *C. bairdi* or *S. fontinalis*.

Cardinal species	Percentage occurrence of associated species with cardinal species						
	<i>Pimephales notatus</i> (Rafinesque)	<i>Hybopsis bimaculatus</i> (Kirtland)	<i>Notropis rubellus</i> (Agassiz)	<i>Hypentelium nigricans</i> (LeSueur)	<i>Etheostoma nigrum</i> Rafinesque	<i>Etheostoma caeruleum</i> Storer	<i>Etheostoma flabellare</i> Rafinesque
<i>M. dolomieu</i>	82	63	55	50	75	70	57
<i>A. rupestris</i>	74	46	34	30	69	52	44
<i>C. bairdi</i>	7	13	2	1	7	7	6
<i>S. fontinalis</i>	7	9	1	2	7	6	5

the three minnows, one sucker and three darters, exhibit a percentage occurrence of similar magnitude with the smallmouth and rock bass and a quite different order of magnitude for the other group of cardinal species, the speckled trout and muddler.

TABLE VI. Five species of fish associated more often with *C. bairdi* and *S. fontinalis* than with *M. dolomieu* or *A. rupestris*.

Cardinal species	Percentage occurrence of associated species with cardinal species				
	<i>Rhinichthys atratulus</i> (Hermann)	<i>Eucalia inconstans</i> (Kirtland)	<i>Chrosomus eos</i> Cope	<i>Margariscus margarita</i> (Cope)	<i>Umbra limi</i> (Kirtland)
<i>M. dolomieu</i>	25	11	3	2	0
<i>A. rupestris</i>	28	15	6	1	6
<i>C. bairdi</i>	78	45	36	24	20
<i>S. fontinalis</i>	61	28	20	18	13

The five species found more commonly with the speckled trout and muddler are listed in Table VI. The percentage occurrence is again similar within each group of cardinal species but quite different between the two groups although the difference is not as striking as in Table V.

Three species were commonly associated with all the cardinal species. They are presented in Table VII.

TABLE VII. Species of fish commonly associated with all cardinal species.

Cardinal species	Percentage occurrence of associated species with cardinal species		
	<i>Catostomus commersoni</i> (Lacépède)	<i>Notropis cornutus</i> (Mitchill)	<i>Semotilus atromaculatus</i> (Mitchill)
<i>Micropterus dolomieu</i>	71	81	79
<i>Ambloplites rupestris</i>	62	76	69
<i>Cottus bairdi</i>	51	37	72
<i>Salvelinus fontinalis</i>	33	29	52

Forty-two additional species were collected; of these, 18 occurred in more than 5 per cent of the stations where a cardinal species was taken as shown in Table VIII.

Frequencies of the remaining 24 species are too small to be significant although it is worthy of note that 15 of them were not taken with the speckled trout and muddler and two, *Pfrille neogea* (Cope) and *Salmo gairdneri* Richardson, were not collected at any of the stations where smallmouth or rock bass occurred. The other species with less than a 5% occurrence with a cardinal species are as follows: *Ichthyomyzon fossor* Reighard and Cummins, *Lepisosteus osseus* (Linnaeus), *Pomolobus pseudo-harengus* (Wilson), *Salmo trutta* Linnaeus, *Cyprinus carpio* Linnaeus, *Notropis atherinoides* Rafinesque, *Notropis hudsonius* (Clinton), *Notropis heterodon* (Cope), *Notropis spilopterus* (Cope), *Ictalurus natalis* (LeSueur), *Noturus gyrinus* (Mitchill), *Anguilla rostrata* (LeSueur), *Fundulus diaphanus* (LeSueur), *Lota lota lacustris* (Walbaum), *Stizostedion vitreum vitreum* (Mitchill), *Percina caprodes* (Rafinesque), *Percina copelandi* (Jordan), *Etheostoma exile* (Girard), *Micropterus salmoides* (Lacépède), *Lepomis cyanellus* Rafinesque, *Lepomis macrochirus* Rafinesque, *Gasterosteus aculeatus* Linnaeus.

TABLE VIII. Association of other species of fish having at least a 5% occurrence with a cardinal species

Associated species of fish	CARDINAL SPECIES			
	Speckled trout	Northern muddler	Rock bass	Smallmouth bass
<i>Pimephales promelas</i> Rafinesque	%	%	%	%
<i>Cottus cognatus</i> Richardson ^a	6	16	12	7
<i>Hybognathus hankinsoni</i> Hubbs	5	1	0	0
<i>Rhinichthys cataractae</i> (Valenciennes)	3	10	5	6
<i>Clinostomus elongatus</i> (Kirtland)	3	10	4	16
<i>Esox lucius</i> Linnaeus ^a	2	11	1	0
<i>Notrus flavus</i> Rafinesque	1	1	9	10
<i>Lepomis megalotis</i> (Rafinesque)	1	0	14	12
<i>Notemigonus crysoleucas</i> (Mitchill)	0	0	7	4
<i>Ictalurus nebulosus</i> (LeSueur)	0	0	8	5
<i>Lepomis gibbosus</i> (Linnaeus)	0	0	12	5
<i>Perca flavescens</i> (Mitchill)	0	0	3	7
<i>Hybopsis micropogon</i> (Cope)	0	1	9	8
<i>Notropis volucellus</i> (Cope)	0	0	10	12
<i>Moxostoma</i> sp.	0	0	17	29
<i>Etheostoma blennioides</i> Rafinesque ^b	0	1	21	29
<i>Etheostoma microperca</i> Jordan and Gilbert	0	1	35	32
<i>Percina maculata</i> (Girard)	0	1	27	40

*Cardinal species in the original study (Hallam, 1954).

^bRestricted to Lake St. Clair drainage in Ontario.

INVERTEBRATES

Many invertebrates were abundantly or commonly taken with all four cardinal species of fish; others occurred infrequently or rarely with both the speckled trout-muddler group and the smallmouth bass-rock bass group. However, a few invertebrates showed marked differences in their frequency of occurrence with the two groups of cardinal species.

Examples of two species of the same genus, two genera of the same family, and two families of the same order, which show such marked difference in their frequency of occurrence with the cardinal species are given in Table IX.

TABLE IX. Selected pairs of categories of invertebrates showing marked difference in frequency of association with the cardinal species.

Cardinal species	Percentage occurrence of associated invertebrates with cardinal species							
	Megaloptera	Trichoptera	Plecoptera	Ephemeroptera	Chauliodes	Helicopsychidae	Rhyacophilidae	Nemouridae
<i>Micropterus dolomieu</i>	9	7	10	4	56	1	23	2
<i>Ambloplites rupestris</i>	7	5	13	3	40	1	12	—
<i>Cottus bairdi</i>	0	18	8	38	36	40	2	20
<i>Salvelinus fontinalis</i>	0	15	0	20	36	60	—	14

*In *Stenonema vicarium* (Walker) group.

There were a number of invertebrates of ubiquitous occurrence. Some others, although not as common, occurred about equally with all cardinal species. A few of these are illustrated in Table X.

TABLE X. Selected categories of invertebrates other than Ephemeroptera associated frequently with all cardinal species.

Cardinal species	Percentage occurrence of associated invertebrates with cardinal species						
	Hyalella	Sialidae	Chironomidae	Simuliidae	Hydropsychidae	Philopotamidae	Limnephilidae
	%	%	%	%	%	%	%
<i>M. dolomieu</i>	70	52	91	49	89	57	24
<i>A. rupestris</i>	71	36	87	47	75	39	23
<i>C. bairdi</i>	36	27	45	31	79	36	20
<i>S. fontinalis</i>	28	23	49	42	78	48	28

The order Ephemeroptera was the only one in which identification to genus or species was usual. Perhaps because of identification to smaller units such as species which have a more restricted habitat than a family, the mayflies show a much greater difference in their frequency of occurrence with the two groups of

TABLE XI. Selected species of Ephemeroptera which are commonly associated with all cardinal species. Three values of percentage occurrence of each invertebrate with a cardinal species are listed, as follows: *upper line*, the total occurrence of the organism; *lower line at left*, the occurrence of the organism in swiftly flowing water; *lower line at right*, the occurrence of the organism in slowly flowing water. Since some invertebrates were taken from both swiftly and slowly flowing water the sum of the last two figures often exceeds the first. The lower-line figures give an indication of the strength of current of the habitat in which the organism is usually found.

Cardinal species	Percentage occurrence of associated species with cardinal species						
	<i>Stenonema canadense</i> (Walker)	<i>Isonychia bicolor</i> (Walker)	<i>Caenis</i> sp.	<i>Baetis frondalis</i> McD.	<i>Baetis pygmaeus</i> Hagen		
<i>Micropterus dolomieu</i>	68 46 55	23 21 3	72 47 62	46 35 31	46 38 27		
<i>Ambloplites rupestris</i>	56 31 48	12 11 2	60 34 54	42 27 34	45 33 30		
<i>Cottus bairdi</i>	37 14 27	16 14 8	28 6 24	27 9 23	23 11 16		
<i>Salvelinus fontinalis</i>	25 10 17	13 11 7	16 5 14	21 7 16	16 7 12		

cardinal species than do most other invertebrates of this study. Five of those which occur more frequently with the centrarchids are illustrated in Table XI and five of those found most often in association with the speckled trout and muddler are presented in Table XII. Some species which do not show such restriction in association with one or other of the cardinal fish groups are given in Table XIII.

TABLE XII. Selected species of Ephemeroptera which are more often associated with the smallmouth bass and rock bass than with the speckled trout and muddler. For explanation, see Table XI.

Cardinal species	Percentage occurrence of associated species with cardinal species				
	<i>Stenonema bipunctatum</i> McD.	<i>Stenonema heterotarsale</i> McD.	<i>Ephoron</i>	<i>Cloeon simplex</i> McD.	<i>Baetis intercalaris</i> McD.
<i>Micropterus dolomieu</i>	39 38	54 46	9 0	42 40	78 71 15
<i>Ambloplites rupestris</i>	27 25	44 32	5 0	36 34	54 48 13
<i>Cottus bairdi</i>	11 5	11 2	0 10	7 1	10 9 1
<i>Salvelinus fontinalis</i>	7 2	9 3	0 6	3 0	9 9 1

TABLE XIII. Selected species of Ephemeroptera which are associated more often with the speckled trout and muddler than with the smallmouth bass and rock bass. For explanation, see Table XI.

Cardinal species	Percentage occurrence of associated species with cardinal species				
	<i>Paraleptophlebia adoptiva</i> McD.	<i>Paraleptophlebia debilis</i> (McD.)	<i>Baetis mcdunnoughi</i> Ide	<i>Baetis brunneicolor</i> McD.	<i>Baetis vagans</i> McD.
<i>Micropterus dolomieu</i>	2 1	3 1	4 2	5 2	8 0
<i>Ambloplites rupestris</i>	1 0	1 1	5 2	4 1	5 1
<i>Cottus bairdi</i>	18 14	20 15	45 32	51 31	49 37 19
<i>Salvelinus fontinalis</i>	20 15	22 11	41 31	55 32	64 53 23

Many other invertebrates identified to species, genus, family or larger division were also collected with the cardinal species. The relative frequency of occurrence (abundant, common, infrequent and rare) of those having at least a 5 per cent frequency with one cardinal species are presented in Table XIV.

In the original study (Hallam, 1954) nine other invertebrates having less than a 5 per cent occurrence with a cardinal species were tabulated. These invertebrates are: the dragonfly genera *Libellula* and *Anax*, the stonefly families Pteronarcidae and Chloroperlidae and the mayflies *Iron pleuralis* Bks., *Siphlonurus*, *Blasturus*, *Ephemerella simplex* McD., and *Heterocloeon curiosum* McD.

DISCUSSION AND CONCLUSIONS

HABITAT

On the basis of average conditions of their habitats the four cardinal species can be grouped into two divisions, the members of each division occupying similar habitats. *S. fontinalis* and *C. bairdi* form one division, and *A. rupestris* and *M. dolomieu* form the other.

TABLE XIV. Relative abundance of categories of other invertebrates having at least a 5 per cent frequency of occurrence with a cardinal species. A—abundant, more than 50%; C—common, 21–50%; I—infrequent, 6–20%; R—rare, up to 5%.

Associated invertebrates	CARDINAL SPECIES			
	Speckled trout	Northern muddler	Rock bass	Smallmouth bass
Mollusca				
Aculidae	R	R	C	C
Planorbidae: <i>Planorbis</i>	R	R	C	C
Planorbidae: <i>Helisoma</i>	R	R	I	I
Lymnaeidae	R	R	I	I
Sphaeriidae	R	R	I	I
Amnicolidae	R	R	I	I
Crustacea	—	—	—	—
<i>Aeselius communis</i> Say	R	R	I	I
Arachnida				
Hydracarina	—	—	I	R
Hemiptera				
Corixidae	R	I	C	C
Notonectidae	—	RRR	I	I
Belostomatidae	—	R	I	I
Nepidae	R	R	I	I
Gerridae	R	R	I	I
Diptera				
Dixidae	R	R	I	I
Tipulidae	R	I	RR	IIR
Tabanidae	R	I	R	I
Coleoptera				
Elmidae, Dryopidae, Psephenidae	II	CII	ACC	ACCC
Hydrophilidae	II	I	C	C
Haliplidae	—	—	—	—
Dytiscidae	—	—	—	—
Gyrinidae	—	—	—	—
Odonata				
<i>Enallagma</i>	R	RR	C	CC
<i>Ischnura</i>	—	RR	I	I
<i>Agrion</i>	R	RR	I	I
<i>Nehallenia</i>	R	RR	I	I
<i>Argia</i>	—	RR	I	I
<i>Boyeria</i>	R	RR	I	I
<i>Aeschna</i>	R	RR	I	I
<i>Basiaeschna</i>	—	—	—	—
Trichoptera				
Psychomyiidae	I	I	I	C
Plecoptera				
Perlodidae	I	I	R	—
Ephemeroptera: Ephemeridae				
<i>Ephemerella guttulata</i> Pic.	I	I	—	R
<i>Ephemerella simulans</i> Walk.	R	I	—	I
<i>Potomantus</i> sp.	—	—	—	—
Ephemeroptera: Heptageniidae				
<i>Stenonema tripunctatum</i> Bks.	II	II	A	AI
<i>S. nepotellum</i> McD.	II	—	IR	II
<i>S. rubrum</i> McD.	—	—	IR	RC
<i>S. vicarium</i> Walker group	C	C	IR	IR
<i>I. humeralis</i> Morgan	II	II	IR	—
<i>Heptagenia pulla</i> Clem.	II	II	—	—
<i>H. hebe</i> McD.	II	II	C	—
Ephemeroptera: Baetidae				
<i>Trichorythodes</i> sp.	I	I	I	CC
<i>Choroterpes basalis</i> Bks.	RR	II	RR	RR
<i>Paraleptophlebia mollis</i> Hag.	RR	II	RR	RR
<i>Ephemerella bicolor</i> Clem. group	RI	II	RR	RR
<i>E. serrata</i> Morgan group	I	II	RR	RR
<i>E. fusca</i> Walker group	I	R	RR	RR

Associated invertebrates	CARDINAL SPECIES			
	Speckled trout	Northern muddler	Rock bass	Smallmouth bass
Ephemeroptera: Baetidae (cont'd)				
<i>E. needhami</i> McD. group	I	I	R	I
<i>Cloeon rubropictum</i> McD.	R	I	I	I
<i>C. triangulifera</i> McD.	I	I	I	I
<i>Pseudocloeon cingulatum</i> McD.	R	R	I	I
<i>P. dubium</i> Walsh	R	R	I	I
<i>P. punctiventris</i> McD.	I	I	I	I
<i>Centroptilum album</i> McD.	I	I	R	I
<i>C. bellum</i> McD. group	I	I	I	I
<i>C. fragile</i> McD.	-	R	I	I
<i>Callibaetis</i> sp.	R	R	R	C
<i>Baetis parvus</i> Dodds.	I	I	I	C
<i>B. cingulatus</i> McD. group	C	C	C	C

A description of the average habitat of the speckled trout-muddler division would be as follows: shaded source waters of low temperature and small volume of flow with many rapids. Rock bass and smallmouth bass, comprising the second division, occurred in open downstream waters of relatively high temperature and large volume of flow with much quiet water.

Results obtained in this study agree with observations of other authors. Radforth (1944) states that "the eastern speckled trout is noted for its particular choice of habitat in which it shows a definite preference for clear, cool highland streams." Shurrager (1932) recorded that he found *G. bairdi* only in headwaters and small streams whereas he found *M. dolomieu* in small and large streams. One of Ricker's (1934) main divisions in his stream classification is the larger warm river, "In the warm rivers trout do not occur, the important piscivorous fishes being pike and centrarchids."

Temperature, shade, volume of flow and other physical conditions of sections of streams are influenced by the distance from the source of the section of stream. Further from the source, streams typically become larger in volume of flow, have a greater percentage of quiet water and a smaller area in shade and are warmer. Any variations from this pattern in the habitat of a species may indicate which of the conditions exercises the most influence on its distribution. The relative position of each cardinal species for each physical condition of the habitat is summarized in Table XV. This table shows that for the first three conditions (distance from

TABLE XV. Summary showing for each cardinal species its relative position in the scale of each physical condition of the habitat. For each of the four cardinal species numbers (1 to 4) have been assigned under each physical condition of the habitat. The numbers 1 to 4 indicate increasing distance from source, decreasing amount of shade, increasing water temperature, decreasing proportion of rapid water and increasing volume of flow.

Cardinal species	Division	Physical Conditions				
		Nearness to source	High % of shade	Low water temp.	High % rapid water	Low volume of flow
Speckled trout	I	1	1	1	1	1
Northern muddler	I	2	2	2	2	2
Rock bass	II	3	3	3	4	4
Smallmouth bass	II	4	4	4	3	3

source, percentage shade and water temperature), the four species occupy the same relative position so that, in effect, there is a succession from species typical of cold, shaded source waters to those typical of warmer, less shaded, downstream water.

Only the speckled trout and northern muddler of Division I occupy the same relative position for all physical conditions listed in Table XV. What factors control the distribution of the cardinal species? Of the conditions listed in Table XV, distance from source and amount of shade likely exercise most control indirectly through their influence on volume and rate of flow and temperature. Shade, of course, is not synonymous with cover which can be important in governing local distribution. Temperature and current are probably the main factors directly controlling the distribution of aquatic organisms in streams. All the cardinal species occur in lakes as well as streams; the trout and muddler are restricted to cool lakes but the centrarchids, although found in such lakes, occur there in the warmer epilimnion. The rock bass and smallmouth bass also occur in lakes that are too warm for the speckled trout and muddler. This suggests that temperature is the main factor controlling the distribution of these cardinal species. In streams, however, other factors such as gradient and volume of flow which together determine rate of flow may be quite important.

In Division I, *S. fontinalis*, which ranks first under all conditions of Table XV, inhabited the smallest, coolest, most heavily shaded source waters having the highest percentage of rapids. *C. bairdi* is found in larger sections further downstream which have less shade, are somewhat less cool and have a smaller percentage of rapids as shown by its relative position in second place for each of the conditions of Table XV. *S. fontinalis* has a low range of selected temperatures, 57-66°F (Fry 1951). Fry notes that "the commonly observed distribution of speckled trout in nature is brought about by a process of temperature selection." It is reasonable to suggest that the species of Division I, *S. fontinalis* and *C. bairdi*, occur in swift, shaded source streams because these waters are cool.

A. rupestris and *M. dolomieu* were located further downstream in larger, quieter, warmer, less shaded waters than were the speckled trout and muddler. *A. rupestris* ranks third and *M. dolomieu* fourth in Table XV for the categories of distance from source, percentage shade and water temperature but their positions are reversed for categories of amount of rapid water and percentage of stations with a large volume of flow. The percentage of still water is much greater for the rock bass than the smallmouth bass! This could be explained as follows: *A. rupestris* is a species usually found in ponds or in pools or other quiet areas of streams so that of the two centrarchids it is not surprising that in this study it was found in locations with more still water and in a greater proportion of locations with a large volume of flow. It might be expected that these locations would be further from the source and warmer than the waters frequented by *M. dolomieu*. The average temperatures of their habitats were similar, that for *M. dolomieu* being a little warmer. We would thus expect to find *A. rupestris* in habitats with much quiet water and *M. dolomieu* in habitats with less quiet water of approximately the same temperature. This could only be achieved if the quiet water habitat

with larger volume of flow of *A. rupestris* (ordinarily subjected to considerable warming) was located closer to the source and was more shaded than the typical habitat of *M. dolomieu*. The fact that this was found to be so seems to indicate that in streams temperature is at least equally important to rate of flow in governing the distribution of these species. Radforth (1944) suggests that the southerly distributed centrarchids are limited in their northward dispersal by their requirement "of a certain summer maximum of temperature to provide them with the necessary conditions for reproduction and growth."

FAUNAL ASSOCIATIONS

CARDINAL SPECIES. It has been shown that on the basis of average physical conditions of the habitat, the cardinal species can be grouped into two divisions. This grouping is substantiated by figures for the degree of association of the cardinal species with each other (Table IV). The smallmouth bass, *M. dolomieu*, and the rock bass, *A. rupestris*, were often associated with each other but almost never associated with the speckled trout, *S. fontinalis*, and the muddler, *C. bairdi*, which latter were frequent associates. There is almost no overlap between these two pairs of species since the 243 places where speckled trout were taken are entirely different and separate from the 222 stations at which rock bass were recorded. Dymor² (1938, 1939) has noted that ". . . *Cottus bairdi* is commonly found associate . . . with the speckled trout," that "it is unusual to find bass and trout occurring in the same water . . .," and that "the rock bass probably occurs naturally wherever the smallmouth bass does."

INDICATOR ORGANISMS. The two divisions of cardinal species have a characteristic fauna associated with them. Some fish and aquatic invertebrates have such wide ranges of tolerance of temperature and current that they are found associated with both groups of cardinal species. Others which show a high degree of association with one group only are probably restricted in their distribution by temperature and/or current. The more closely their environmental requirements coincide with those of a cardinal species, the higher will be their degrees of association with this species.

Before turning to associated fish and invertebrates, consideration, in more detail, will be given to associations between cardinal species in order to expand the idea of indicator organisms. It is possible that the best indicator of one cardinal species may be one of the other cardinal species. Data for the cardinal species, show not only how often species A occurred with species B, but also how often species B occurred in the collections containing species A. For examples, *Cottus cognatus* Richardson, which in the original study (Hallam, 1954) was also a cardinal species, occurred at only 5% of the stations where the speckled trout was collected, but the latter occurred in 12 of 14 or 86% of the collections containing *C. cognatus*. The presence of speckled trout would thus give little evidence of the presence of *C. cognatus* or even of waters suitable for the latter. The presence of *C. cognatus*, however, would be an almost sure indication of the presence of speckled trout or of a habitat suitable for the latter. *C. cognatus* might thus be a "sure" indicator of speckled trout but it cannot be considered the best

or even a good indicator. It has a very restricted distribution in Ontario and if we were to rely on its presence as an indicator of trout waters we would find very little indeed. The northern muddler, *C. bairdi*, is a more useful, though less certain, indicator of waters inhabited by or suitable for speckled trout. The data obtained in this study show that the smallmouth bass is an excellent indicator of rock bass but the latter is only a fair indicator of the former throughout an area in which both are generally distributed.

Data for other organisms associated with the cardinal species show only how often an organism occurred with a cardinal species and does not show the percentage occurrence of each cardinal species with each organism. There may, therefore, be some organisms which, like *C. cognatus*, are rare but which occur most of the time in association with one of the cardinal species. The data as tabulated and analysed do not show such "sure", but not necessarily useful, indicators. This leads to a consideration of the criteria of an indicator organism useful for determining the presence of, or waters suitable for, a given species of fish.

1. The organism must be commonly associated with the species of fish of which it is an indicator, throughout the range of the fish.
2. The indicator organism may have a high degree of association with fish which are themselves frequently associated with a certain species of fish but it must be absent, rare or infrequently taken with fish that are seldom associated with the particular species of fish.
3. Some mayflies most typical of cool, trout waters are present as nymphs in early spring in waters that in midsummer are too warm for all stages except the diapausing eggs (Ide, 1935) and these waters are then too warm for speckled trout. Such a species would be less satisfactory as an indicator of speckled trout than one which was absent at all times from these warmer waters.
4. Certain organisms, such as some species of the stonefly genus *Nemoura*, are found in cool waters suitable for speckled trout but are useful as indicators only in the spring, since they are early-emerging forms which are in evidence as nymphs only at the very beginning of the summer season (Sprules, 1947).

ASSOCIATED FISH. Of the other species of fish considered in detail most were associated more often with one group of cardinal species than another. If a certain species was found most often with the rock bass or with the northern muddler, then in general its next highest percentage occurrence was with the smallmouth bass or the speckled trout, respectively, the species with habitats most similar to the first two species.

Many species were associated frequently with the two centrarchids. The 7 most significant species were presented earlier in Table V. In a survey of the lower Credit River, Dymond *et al.* (1929) found 6 of the 7 species listed in Table V as occurring with the smallmouth and rock bass. The one species of Table V not found in that survey was *Hybopsis biguttatus*, although the closely related *H. micropogon* (Cope) did occur there in association with the centrarchids. *Pimephales notatus*, *Etheostoma nigrum* and *E. caeruleum* all showed higher

percentage occurrences with these cardinal species than the two cardinal species did with each other, but all three had a small but not insignificant occurrence with the speckled trout and northern muddler. Therefore, *Notropis rubellus* and *Hypentelium nigricans*, which had very small occurrences with the trout and muddler, would appear to be better indicators for the two centrarchids. A species of restricted distribution, *Etheostoma blennioides* (found only in the St. Clair River drainage, in Ontario), had a high frequency of occurrence with the small-mouth and rock bass in the Thames River and its tributaries and might be very useful as an indicator of waters suitable for these species in the region throughout which it is distributed.

The five species found more commonly with the speckled trout and northern muddler are listed in Table VI. None of these five associated species was found in the study of the lower Credit River by Dymond *et al.* (1929). However, Ricker (1934) in his study of a hard-water trout stream, the Mad River, found six species other than speckled trout. In addition to the muddler, he found all of the species of Table VI except *Umbrina limi*. None of the species of Table VI is as good an indicator of speckled trout and the muddler as these latter two are of each other. *Margariscus margarita*, the pearl dace, appears to be the next most useful as an indicator of these two cardinal species.

The three species commonly associated with all cardinal species are *Semotilus atromaculatus*, *Notropis cornutus*, and *Catostomus commersoni*. Ricker (1934) notes that the fish fauna of soft-water trout streams includes *N. cornutus* and *C. commersoni*. These two species also were taken with the centrarchids in the study of the lower Credit River, Dymond *et al.* (1929). The only species collected in the surveys which could be considered to be as much at home in waters inhabited by rock bass and smallmouth bass as in those containing northern muddler and speckled trout, is the creek chub or horned dace, *Semotilus astromaculatus*, for the latter was found in more than half the locations at which all four cardinal species were taken.

ASSOCIATED INVERTEBRATES. As with fish, temperature and current are the main factors controlling the general distribution of invertebrates in streams. Ide (1935) found that "temperature of the water sets limits to the distribution of the mayflies within which are other limits determined by the rate of flow of the water, type of bottom and vegetation." For three orders of insects, Trichoptera, Ephemeroptera and Plecoptera, Sprules (1947) found that their distribution in streams is the results of the complex interaction of many environmental factors including temperature, type of bottom and rate of flow.

In general, invertebrates of lentic habitats had the highest frequency of occurrence with the centrarchids whereas those of lotic habitats occurred more frequently with the speckled trout and muddler. However, there were numerous exceptions to the latter and a few to the former thesis and it is such exceptions which may be especially useful as indicator species.

The predominant occurrence of invertebrates other than insects with the rock bass and smallmouth bass is probably owing to the emphasis placed on collecting certain insects in the surveys in which the trout and muddler were most often collected. It is not surprising, however, that representatives of the Mollusca and

Crustacea have a higher frequency of occurrence with the cardinal species of habitats with much still water, since these invertebrates are more abundant in this type of habitat. An invertebrate of lentic habitat will also be collected in some habitats that are classed as lotic. The ubiquitous amphipod *Hyalella* of lentic habitats was found more often in lentic than lotic habitats, but was taken in a greater proportion of rapid water habitats with the centrarchids of slower waters than it was with the trout and muddler of swift waters. Figures for the genus *Caenis* (Table XI) show that a similar phenomenon can be found among the mayflies. The reason for this may be that in some of the warmer still waters where the centrarchids are found oxygen requirements are not always met, and *Hyalella* (or *Caenis*) therefore occurs relatively more often in moving waters in which the dissolved oxygen will be nearer saturation. Some forms, like the isopod *Asellus communis*, are tolerant of low oxygen and are often found in situations of mild pollution. This isopod occurred much more often with the centrarchids than with the speckled trout or muddler, probably because the centrarchids were taken from waters running through agricultural country where cattle have access to the streams.

Among the insects, the Coleoptera, Hemiptera and the Diptera, except for the family Tabanidae, show a preponderant occurrence with the centrarchids. That closer attention to smaller taxonomic units will reveal more indicators are shown by the family Sialidae of the order Megaloptera. The Sialidae, as a whole, was found commonly with the speckled trout and muddler and the centrarchids. However, one genus, *Chauliodes*, was taken much more often with the cardinal species of cold water. Another genus, *Corydalus*, although confined to rapids, was found only with the centrarchids, the cardinal species of warmer waters which have the highest percentage of still water. It would seem to be restricted in its distribution by temperature, its presence in a stream indicating water too warm for speckled trout. The dragonfly genus *Enallagma*, where it occurs in streams, appears to be restricted to and thus indicative of warm waters with a low percentage of rapids. The caddisflies studied were found in evenly-flowing or rapid waters but the family Helicopsychidae occurred only with the centrarchids and so would seem to be characteristic of warmer water. The family Rhyacophilidae occurred more often in the cooler trout waters.

Sprules (1947) found a decrease in the number of species of Plecoptera downstream and concludes "that this group is made up primarily of species adapted as cold-tolerant." Within the group he found the genera *Leuctra* and *Nemoura*, of the family Nemouridae, among those most abundant in upstream waters. These stonefly genera are the only invertebrates so far considered which appear to satisfy most of the criteria of useful indicators. Their presence would indicate waters inhabited by or suitable for the speckled trout. Some species of these genera are early summer forms while others emerge in late summer. The former would be useful as indicators in the spring, the latter being the only ones useful in July and August.

In the Ephemeroptera, many species and genera show striking differences in their frequencies of occurrence with the groups of cardinal species. Only in this

order was identification to species usual and this probably accounts for the good separation of the mayflies into those typical of warm quieter water inhabited by the centrarchids and those typical of colder more rapid water in which the speckled trout and muddler live. Whitney (1939) found that "Nymphs from slow or still water have a greater resistance towards high temperatures than comparable nymphs from swift waters. This is correlated with the more extreme conditions of temperature which are known to exist in slow and still water." Many closely related species and genera can be separated on a basis of their physiological requirements as well as by their morphological characteristics. Ide (1935) found that, "Very closely related species in a genus are apparently seasonally isolated by the temperature of the water," which he states "may be explained by the different growth rates and different thresholds of development and lethal temperatures."

It is interesting to speculate on the relative importance of the influence of temperature and current on the association of the invertebrates, especially the mayflies, with the different cardinal species. For the many species which occur more often with the centrarchids than with the speckled trout and muddler, the great majority have a percentage occurrence in the following order: greatest with smallmouth bass, next with rock bass, followed by northern muddler and least with speckled trout, which follows the relative order of average water temperature for these species. However, for the species which occur more often with the speckled trout and muddler than with the centrarchids, the great majority have a percentage occurrence in the following order: greatest with speckled trout, next with northern muddler, followed by smallmouth bass, and least with rock bass. This order does not follow that for temperature but rather that for proportion of quiet water. The species which occur frequently with speckled trout and muddler are those which can tolerate or require cool temperature. If they are seldom taken with centrarchids they are probably restricted to cool water. Why do they almost invariably have a slightly higher percentage occurrence with the smallmouth than with the rock bass even though the former is found in waters on the average a little warmer? Perhaps it is because in the water in which rock bass are found the lack of oxygen becomes a limiting factor. The waters in which smallmouth bass are found are slightly warmer and therefore would contain slightly less oxygen if they had the same proportion of still water. These waters, however, have a much higher proportion of rapids and would therefore have a greater proportion of water with oxygen content near saturation.

That these factors may be operating can be seen in a rapid water species which is found about as commonly with the centrarchids as with the trout and muddler, namely, *Isonychia bicolor*. This mayfly obviously has a wide temperature tolerance for it occurred commonly with both groups of cardinal species. As is shown by its occurrences in rapid and still water habitats of Table X, it was taken in the case of each cardinal species from many more rapid than still water habitats. However, the proportion of its rapid to still water habitats was much higher in its occurrence with the centrarchids than it was with the speckled trout and muddler. This suggests that in the cool waters of the trout and muddler, *Isonychia bicolor*

is able to find in the quieter sections conditions suitable for existence including a sufficiently high level of oxygen.

Often two species of the same genus will have similar percentage occurrences with the cardinal species, but one will be predominantly from lentic habitats, the other from lotic habitats. An example of such a pair is *Baetis brunneicolor* and *B. mcdunnoughi*. Both were common or abundant with the speckled trout and muddler but occurred rarely with the centrarchids. Of these two species of mayflies we would expect and we do find that *B. brunneicolor*, the lotic form, has a slightly greater percentage occurrence with the speckled trout and muddler than has *B. mcdunnoughi* of lentic waters; so that the latter, even though the figures for its percentage occurrence do not at first glance justify the conclusion, may be as good or better indicator of trout water than *B. brunneicolor*.

Some genera or species of mayflies do not fulfill all the criteria of useful indicators but they appear to be characteristic of waters inhabited by one of the groups of cardinal species. Those typical of waters inhabited by the centrarchids include the genera *Potamanthus* and *Ephoron* and the species *Stenonema rubromaculatum*, *S. heterotarsale*, *S. bipunctatum*, *Cloeon simplex* and *Centroptilum fragile*. Those typical of trout waters include *Heptagenia pulla*, *Stenonema vicarium* group, *Paraleptophlebia adoptiva*, *P. debilis* and *Ephemerella needhami*.

Certain species of the mayfly genus *Baetis* together with the aforementioned stonefly genera *Leuctra* and *Nemoura* (in springtime) are the organisms which can be considered excellent indicators. *Baetis intercalaris* is a species of rapid water but its preponderant occurrence with the smallmouth and rock bass as compared with the speckled trout and northern muddler shows that it is an indicator of warm waters suitable for the centrarchids. Even where it has a moderate occurrence with the speckled trout as in 14 of the 99 stations where the trout were taken on the Saugeen River, it is largely from warmer downstream waters as 11 of these 14 stations were located more than ten miles from the source. *Baetis mcdunnoughi*, *B. vagans* and *B. brunneicolor* emerge from this study as the best individual indicators of waters suitable for the speckled trout and muddler. That the figures for total occurrence of these three species with the cardinal species are reliable is borne out by figures for occurrence of these species in lentic or lotic habitats. *B. mcdunnoughi* is found in evenly flowing waters. *B. brunneicolor* inhabits the vegetation at the edge of rapid water and *B. vagans* is found right in the rubble of the rapids. Figures for these species (see Table XIII) show in the same order an increasing percentage occurrence in fast waters.

B. vagans and *B. brunneicolor* have an occurrence with speckled trout of 64% and 55%, respectively, and with rock bass of 3% and 4%, respectively. For these two species it was determined how often either one (*i.e.*, at least one of them) occurred with the cardinal species. The occurrence of either one with speckled trout was 82% and with rock bass 5%. This leads to the important conclusion that reliance should not be placed on any one indicator but rather on a number of indicators together. If a section of stream is found to contain several indicators typical of trout waters and to lack the best indicators of bass waters, then it would almost certainly be a place suitable for the speckled trout and the muddler.

SUMMARY

1. Selected stream report forms of streams in Ontario surveyed by the Department of Planning and Development from 1946 to 1952 form the basis of this study.

2. Data of physical conditions of streams and associated fauna for four species of fish were chosen for analysis. These species are *Salvelinus fontinalis*, the eastern speckled trout, *Cottus bairdi*, the northern muddler, *Micropterus dolomieu*, the smallmouth bass and *Ambloplites rupestris*, the rock bass, and are designated the cardinal species of fish.

3. *M. dolomieu* and *A. rupestris* were found most often more than 10 miles from the source. *C. bairdi* was collected closer to the source than the above and *S. fontinalis* usually occurred less than 3 miles from the source.

4. The speckled trout and muddler were taken from waters which were on the average more than 8 Fahrenheit degrees (4.5 Centigrade degrees) colder than those for the rock bass and smallmouth bass.

5. Shaded source waters of small volume of flow and swift current are the typical habitat of the speckled trout and the muddler. Sunny downstream waters of large volume of flow and slow current are more typical of the habitats of the two centrarchids.

6. On the basis of physical conditions of the habitat, the four species can be grouped into two divisions. *S. fontinalis* and *C. bairdi* form one division and *M. dolomieu* and *A. rupestris* form the other.

7. More species of fish are associated with the cardinal species of downstream waters than with the cardinal species of headwaters.

8. The grouping of the cardinal species on the basis of average physical conditions of the habitat is substantiated by their degree of association with each other.

9. Thirty-three species of fish had an occurrence of 5% or higher with one of the cardinal species. Twenty of these were associated most often with the centrarchids, 7 most often with the trout and muddler, and 6 were taken about as often with both groups of cardinal species.

10. Twenty-four other species of less than 5% occurrence are listed.

11. The percentage frequency of a few invertebrates and the relative frequency of many others with the cardinal species are presented.

12. Temperature and current probably exercise the most influence on the distribution of the cardinal species.

13. Criteria of indicator organisms are listed. A "sure" indicator is not necessarily a good one because it may be rare.

14. The Mollusca, Crustacea and Arachnida considered in this study are organisms of slowly-flowing or still water and have their greatest percentage occurrence with the smallmouth and rock bass.

15. In general, members of the Hemiptera, Diptera, Coleoptera and Odonata were found most frequently with the centrarchids. The genus *Corydalis* of the Megaloptera seems characteristic of warmer bass waters.

16. Most caddisfly genera were commonly represented with all cardinal species. The family *Helicopsychidae* and most species of *Rhyacophilidae* appear to be typical of bass and trout waters, respectively.

17. In the stonefly family *Nemouridae*, the genera *Leuctra* and *Nemoura* indicate waters suitable for the muddler and speckled trout.

18. Many species of mayflies are characteristic of one of the divisions of cardinal species. *B. intercalaris* is an indicator of smallmouth and rock bass waters; and *B. vagans*, *B. brunneicolor* and *B. mcdunnoughi* indicate waters suitable for or inhabited by the speckled trout and northern muddler.

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Active Respiration of Fish in Relation to Ambient Concentrations of Oxygen and Carbon Dioxide¹

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ABSTRACT

The oxygen consumption at a steady rate of activity maintained by a mild electric stimulus was measured for *Salvelinus fontinalis* (Mitchill), *Catostomus commersoni* (Lacepede), *Ameiurus nebulosus* LeSueur, *Cyprinus carpio* (Linnaeus) and *Carassius auratus* (Linnaeus) in the presence of various combinations of oxygen and carbon dioxide. At a given level of oxygen the logarithm of the rate of oxygen consumption decreases linearly with the concentration of carbon dioxide. The linear relation so found is characteristic of a given species and the sensitivity to carbon dioxide decreases with increasing acclimation temperature. The oxygen concentration determines the level of active oxygen consumption in the absence of carbon dioxide and when the concentration approaches the lower lethal limit the effect of the addition of carbon dioxide is enhanced. The data found for these species for active metabolism was combined with data in the literature for resting metabolism to give estimates of the metabolism available for activity. The respiratory sensitivities of the four species were further compared by considering the estimated combinations of oxygen and carbon dioxide required to bring about asphyxiation in both flowing water and sealed containers. The ability of the blood of the fish to take up oxygen in the presence of carbon dioxide shows no direct relation to the ability of the fish to transport oxygen to the external medium under similar circumstances.

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INTRODUCTION

IT IS WIDELY ACCEPTED that the concentrations of dissolved respiratory gases often influence the metabolic rate of aquatic animals. The relation of the concentration of dissolved oxygen in the water to aquatic respiration has been frequently studied. For fish the limits of oxygen concentration below which activity is limited have been more or less satisfactorily established for a number of species (Fry and Hart, 1948; Graham, 1949; Gibson and Fry, 1954; Job, 1955; Hart, in Fry, 1957; Ferguson, in Fry, 1957). The general relation is that when the oxygen concentration is decreased below a critical level the fish is unable to satisfy its needs for oxygen and thus the oxygen consumption becomes dependent on the oxygen concentration of the medium (see the reviews of Tang, 1933; von Ledebur, 1939; Zeuthen, 1955.)

In contrast, very little work has been carried out on oxygen consumption in the presence of carbon dioxide (Hall, 1931; Meyer, 1935; van Dam, 1938; Black, 1945; Basu, 1951 and 1952.) For this reason the present work was undertaken and embraces a systematic study of the respiration of fish stimulated to activity in the presence of various concentrations of oxygen and carbon dioxide.

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MATERIALS AND METHODS

Five species of freshwater fish belonging to 4 families and characteristic of widely different habitats were selected for the present investigation. These species were the speckled trout (*Salvelinus fontinalis*), white sucker (*Catostomus commersoni*), carp (*Cyprinus carpio*),

goldfish (*Carassius auratus*), and the brown bullhead (*Ameiurus nebulosus*). Of these, the trout and sucker are typical of cold, well oxygenated water and are characterized by blood having a low oxygen affinity and a high carbon dioxide sensitivity. Carp, goldfish and bullhead are inhabitants of warm water and are characterized by having a high oxygen affinity and a low carbon dioxide sensitivity of the blood. Goldfish were included since they are widely available all over the world as a laboratory animal.

The speckled trout were obtained from the hatchery at Chatsworth, Ontario. All other species were obtained from natural waters from various parts of the province mostly by seining and in a few cases by gill or hoop net.

Upon arrival at the laboratory, the fish were transferred to outside cement holding tanks (24 x 72 x 18 inches) at a temperature similar to that from which the fish had been taken. Records of the mortality of fish in the holding tanks were kept and in the case of heavy mortality, the stock was supplemented with a fresh catch. The tanks were supplied with running well water, aerated and filtered through a sand filter.

TABLE I. Analysis of the filtered water supply, Laboratory for Experimental Limnology, Maple, Ontario, 1955-56. All analyses reported in parts per million unless otherwise stated. (Temperature, dissolved oxygen, carbon dioxide and pH were determined by the author. Total phosphorus, total nitrogen, specific resistance and specific conductivity were determined by Miss A. Papson, Department of Lands and Forests, Maple, Ontario. The rest were analysed by the Ontario Department of Public Health. See Brett (1952) for an analysis of the unfiltered water at this Laboratory.)

Items	Values	Items	Values
Temperature	9°C	Potassium as K	1.26
Colour in units	3.0	Sulphate as SO_4	30.60
Turbidity in units	2.3	Phenol equivalent	0.80
Dissolved oxygen	11.33	Nitrate as NO_3	Nil
Carbon dioxide	2.0	Nitrite as NO_2	Nil
pH	8.0	Ammonia as NH_3	Nil
Hardness as CaCO_3	269.2	Silica as SiO_2	21.20
Alkalinity		Aluminium as Al	Nil
Total	224.	Copper as Cu	Nil
Carbonate	214.	Phosphate as PO_4	0.045
Bicarbonate	10.	Total phosphorus	0.0013
Iron as Fe	0.02	Total nitrogen	Trace
Iron as Fe_2O_3 (oxide)	0.11	Solids: Total	282.
Chloride as Cl	2.70	Dissolved	278.
Calcium as Ca	82.30	Suspended	4.
Magnesium as Mg	10.30	Specific resistance	2540.
Sodium as Na	2.50	5-day B.O.D.	Less than 0.2

In 1954 the fish were fed "Red Gil" fish food supplied by Burns and Co., Calgary. The composition of this food has been given by Job (1955). Unfortunately the company stopped preparing this material in 1954. After a short interim period of feeding with ground liver the author developed a mixture of 48% liver, 5% brewers' yeast, 2% salt and 45% "pre-starter turkey mash." The mash was obtained from the Master Feed Company of Toronto.

A bi-weekly salt bath (1% NaCl) was given to the fish in the outside holding tanks as a preventive measure against infection by fungus, bacteria or other external parasites. Salt treatment alone was not sufficient in the case of the bullheads, which usually congregated in one place in the tank, mostly in corners. They occasionally injure each other by their spines and become infected. Treatment with acriflavin (2 mg/l) was found satisfactory. Occasional treatment with acriflavin was found to yield a better result even in the scaled fish. Pickford (1952) observed that prolonged treatment with acriflavin retarded the growth of *Fundulus heteroclitus* and accordingly no prolonged treatments were used.

ACCLIMATION

Healthy fish were selected from the stock in the outside holding tanks, about 3 to 4 weeks after the initial transfer of the fish to the laboratory. Under no circumstances were

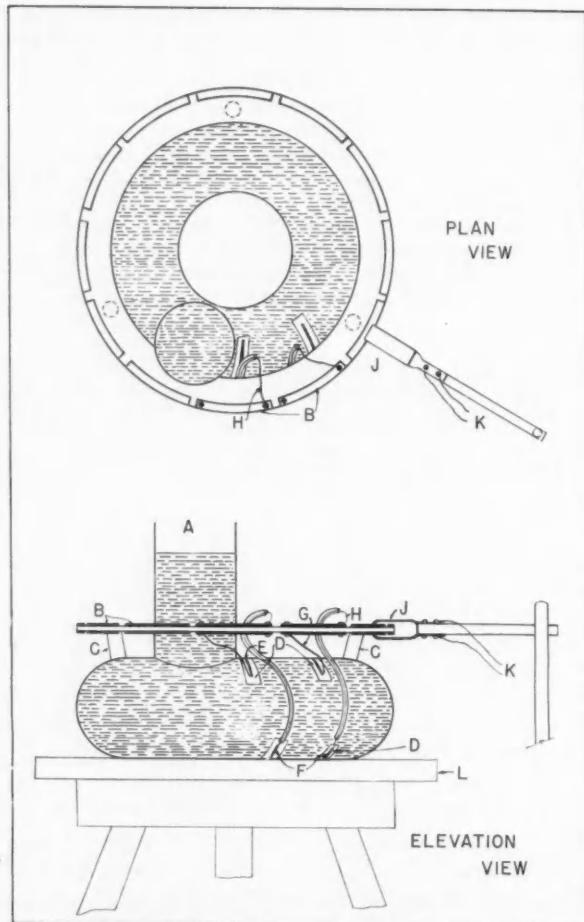


FIG. 1. Rotating chamber for active metabolic rate experiments. Dimensions: Outer diameter 12 inches, inner $4\frac{1}{2}$ inches. Bore approximately $3\frac{1}{2}$ inches. Capacity 5 liters. A. Opening, 5 inches high and $3\frac{1}{2}$ inches diameter. B. Commutator. C. Support. D. Aluminium electrodes. E. Upper electrodes. F. Lower electrodes. G. Connection of lower plate of commutator. H. Connection of upper plate of the commutator in plastic tubing. J. Pair of copper brushes. K. Connection of brushes to power source. L. Turntable.

samples selected from the stock until the fish had been feeding well for at least a period of 15 days. The selected fish were then moved into indoor tanks (capacity 200–300 l) for acclimation.

Procedures laid down by Belding (1929), Brett (1941), and Fry and Hart (1948) were followed for acclimations, which were to 10, 15, 20 and 30°C. The temperature in the acclimation tanks was increased one centigrade degree per day, until the desired level of

temperature was reached. The fish were then kept at that temperature for not less than one month before experiments were performed. To obtain the desired temperature, the tap water was passed through a number of glass heaters made up of Pyrex tubing (Job, 1955). The water in the tank was vigorously aerated through carborundum air diffusers. The oxygen partial pressure was maintained around 92% air saturation, the carbon dioxide was between 1 and 2 ppm and the pH varied from 7.80 to 7.95. In one or two cases considerable fluctuation of temperature due to the deposition of calcium in the tubing occurred in the 30°C acclimation tank. After a fluctuation of less than $\pm 2^{\circ}\text{C}$ the fish were held at the restored constant temperature for at least 24 hours before experiment. If the fluctuation was more than $\pm 2^{\circ}\text{C}$, the time allowed for correcting the acclimation was a minimum period of one week.

An occasional 1% salt bath was given to the fish during the period of acclimation. No other prophylactic measures, however, were adopted.

APPARATUS

An improved rotating chamber developed from types used by Fry and Hart (1948) and Job (1955) and essentially the same as that figured by Fry (1957) was used (Fig. 1). The respiration chamber is doughnut-shaped, moulded from plastic with an inlet tube at the top through which fish may be introduced and samples for analysis may be removed. The chamber is thus closed to the atmosphere except for the area of the inlet tube. Blank runs showed that the gas exchange through this open inlet is negligible. Allowing the tube to remain open obviated the difficulty that Job (1955) had of excess pressure developing when closing the chamber and sealing it by means of an inflated rubber gasket. The chamber was mounted on a wooden circular disc as an additional support for placing it on the turntable, which was the same one used by Fry and Hart (1948).

The stimulator consisted of thin aluminium electrodes each covering about a radial quadrant of the toric surface measuring $3 \times 1/6$ inch mounted on a $3 \times 1/2$ inch plastic plate fixed on the top and bottom of the chamber. Twelve pairs of such electrodes were mounted radially about 2 inches apart. The electrodes were connected by aluminium wire to plates of a commutator. The commutator consisted of a plastic annular ring $1\frac{1}{2} \times \frac{1}{2}$ inch mounted on the top of the chamber supported on three solid plastic rods 3×1 inch long, placed 8 inches apart. On each side of the commutator 12 aluminium plates measuring $2\frac{1}{8}$ inches long, $3/10$ inch wide and $1/10$ inch thick were fixed with $3/10$ -inch gaps between successive plates. The upper series of electrodes of the chamber were connected individually to the upper plates of the commutator, the lower electrodes were all linked together by aluminium wire and to each of the lower plates. To establish contact with the power source, a pair of copper brushes mounted on a plastic plate was then fixed on a stand. A 40-watt light was fixed near the chamber just in advance of the position where the electrodes become charged to serve to identify the charged segment for the fish. A low voltage alternating current (3 volts, 60 cycle) was transmitted to the commutator through the pair of brushes.

METHOD OF OBTAINING DIFFERENT CONCENTRATIONS OF OXYGEN AND CARBON DIOXIDE IN WATER

The apparatus used for obtaining a continuous supply of water with different oxygen and carbon dioxide concentrations is shown in Fig. 2. The apparatus is the column described by Shepard (1955) for removal of dissolved oxygen from the water by displacement with nitrogen, modified by additional reservoirs in which CO_2 or further oxygen could be added to the water. Two 4-foot glass columns of 4 inches diameter packed with glass marbles were fitted one above the other to obtain a large flow of experimental water. To prevent blockage of the outlet, an aluminium screen was placed at the bottom of each column. To simplify the description of the apparatus, the top column will be designated as Column 1, the bottom as Column 2 (items 7 and 8 in Fig. 2). An inlet tube to draw the water from a constant head reservoir and an outlet tube for the escapement of the gas were fixed at the top of Column 1. To facilitate the introduction of the supply water and the escapement of the gas, no

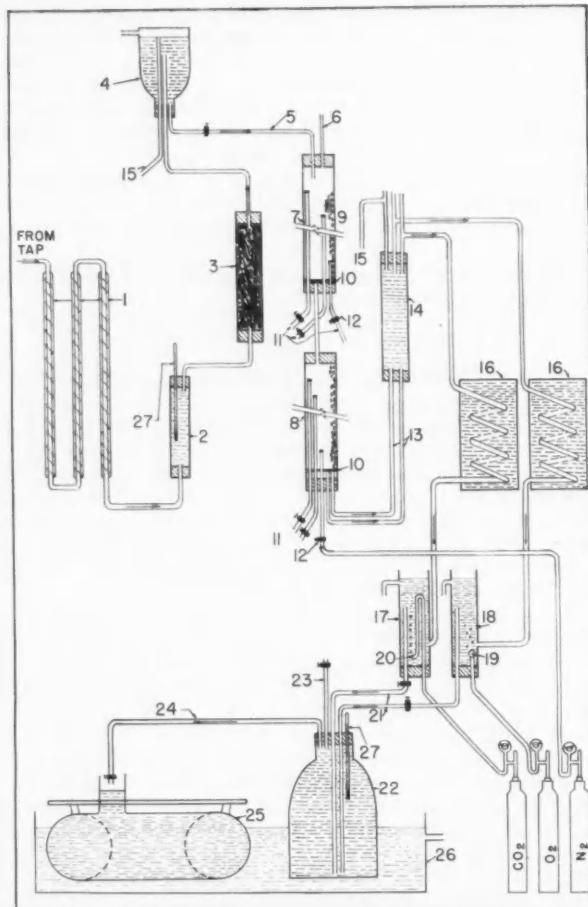


FIG. 2. Arrangements for continuous supply of water with different O_2 , CO_2 , and temperatures. 1. 500-watt Pyrex heaters. 2. Temperature of supply water. 3. Glass wool filter. 4. Constant head reservoir. 5. Inlet to fractionating columns. 6. Gas escape. 7, 8. O_2 fractionating columns. 9. Glass marbles. 10. Aluminium screen. 11. Nitrogen inlets. 12. Screw cocks. 13. Inlet to 14. 14. Constant head reservoir. 15. Overflows. 16. Heat exchangers. 17. CO_2 equilibrating column. 18. O_2 equilibrating column. 19. O_2 diffuser-outlet. 20. CO_2 outlet. 21. Intake to 22. 22. Mixing bottle. 23. Air trap. 24. Outlet tube. 25. Respiration chamber. 26. Constant temperature bath. 27. Thermometers.

marbles were put in its upper four or five inches. The inlet tube reached about 2 inches below the cork, while the gas outlet tube reached just below the cork to prevent interference between the incoming water and the outflowing gas. Column 2 was completely filled with marbles. Provision was made for injecting nitrogen at various levels of the column by introducing glass tubes of different lengths from the bottom of each column. The upper ends of these glass

tubes were protected by aluminium screen guards to prevent blockage of the passage of the gas. The free ends of these inlet tubes outside the column were closed by using pressure tubing and screw cocks.

Two outlet tubes $\frac{1}{4}$ inch in diameter were provided to draw off the accumulated water from the bottom of Column 2. These outlet tubes were connected to a constant head reservoir. By raising or lowering this reservoir, the head of water in Column 2 could be controlled. This constant head reservoir was provided with outlets to supply water to heat exchangers and to two small columns for charging the water with a definite amount of oxygen and carbon dioxide respectively. These equilibrating columns were made of plastic tubing $1\frac{1}{2}$ feet \times 2 inches). They were mounted a foot and a half below the bottom of Column 2 and different rates of water flow through them were obtained by changing the head in Column 2 for the supply of water to the mixing bottle. In each of these equilibrating columns, two $\frac{1}{4}$ -inch plastic tubes were fixed on opposite sides. The lower one was an inlet tube from the heat exchanger situated 3 inches above the bottom of the column. The other, inserted $\frac{1}{2}$ inch from the top, served as an overflow. Each of these columns was further provided with two tubes from below. One was for withdrawing water for the mixing bottle and reached three-quarters of the way up the column. In order to avoid atmospheric contamination, water was not drawn off from the top. The other tube for introducing either oxygen or carbon dioxide entered 2 inches above the bottom. In the oxygen equilibrating column, a diffuser was attached to the short or bottom tube.

A pyrex bottle of 15 liters capacity for mixing water charged with oxygen and carbon dioxide from the equilibrating columns was fitted with two inlet tubes reaching almost to the bottom, an outlet tube, a tube for trapping gas bubbles and a thermometer.

The water supply for the column was drawn from the tap and heated three to four degrees warmer than the temperature at which the experiment was to be performed. The reason for heating the water to a slightly higher temperature and then cooling it down to the desired temperature was to avoid supersaturation of total gases after the final addition of oxygen and carbon dioxide. Otherwise bubbles evolve in the respiration chamber. This heated water was then passed through a glass wool filter to remove any precipitated iron or calcium carbonate, that had been formed as a result of the heating. The water finally passed to the constant head reservoir from which the columns are fed.

Depending on the nature of the experiment performed, either compressed air or nitrogen was bubbled through columns 1 and 2. In the case of the experiment with air-saturated water, air was allowed to bubble through the bottom of Column 2, while the water was slowly cascaded from Column 1. To obtain water having various concentrations of oxygen below air saturation, nitrogen was introduced at particular depths of the column through the appropriate glass tube. To obtain practically oxygen-free water (0.1 ppm from 10.5 ppm at 12°C) the nitrogen was introduced at the bottom of Column 2 at the rate of 350 cc/minute, while the water flowed at the rate of 3 liters/minute, into the top of Column 1. For 20 and 30°C , the flow of nitrogen was 260 and 200 cc/minute respectively.

Various carbon dioxide concentrations were obtained in the carbon dioxide column by regulating both the flow of the inlet water and the rate of flow of carbon dioxide and was standardized by preliminary experiments at different temperatures.

To obtain any desired concentration of oxygen and carbon dioxide, the water from the two columns was mixed in the mixing bottle.

Checks at intervals of 15 minutes for one hour were made on the outlet water to see that the concentrations of oxygen and carbon dioxide maintained a steady rate. The temperature of the water supply to the columns was regulated within $\pm 0.1^{\circ}\text{C}$. Any gas which accumulated in the mixing bottle was released periodically through the air trap at the top. The mixed water was then cooled to the desired temperature in the heat exchanger.

CHEMICAL ANALYSIS

Procedures recommended in standard methods American Public Health Association (1955) were followed. The sodium azide modification of the Winkler method was used for the estimation of dissolved oxygen as a precautionary measure against nitrite interference, although

periodic checks on the water samples both before and after the experiment in the respiration chamber showed the absence of nitrite. A 50 ml sample of water was used for dissolved oxygen estimation and samples were titrated by N/100 standard thiosulphate prepared fresh daily from the stock solution. Sodium hydroxide was added as preservative to the stock solution. For estimation of supersaturation values of oxygen, thrice the quantities of the reagents used in the Winkler's method were used to fix the dissolved oxygen. This procedure was adopted on the basis of experiment. On the other hand no extra manganous chloride was needed when determining oxygen in the presence of high carbon dioxide as van Dam (1935) inferred was necessary.

Free carbon dioxide was estimated by titration with N/22 sodium carbonate stored in paraffin-coated pyrex bottles using phenolphthalein as an indicator. This titrated value was checked against the calculated value obtained by the graphic determination of carbon dioxide (Moore, 1939) based on pH and carbonate equilibria. Moore presented a graph based on data collected at 25°C and suggested temperature corrections to obtain values at other temperatures. Temperature corrections were calculated and were found to be 1% for every 5 Centigrade degrees from 20 to 30°. For calculating the estimated value these corrections were incorporated.

Total methyl orange alkalinity, expressed at parts per million CaCO_3 , was determined by titration with N/50 H_2SO_4 using methyl orange as an indicator.

The pH of the samples was measured with a glass electrode using a Beckman Model H-2 electric pH meter.

To test for appreciable organic pollution of the water in the respiration chamber during the experimental run, determinations of the 5-day 20°C B.O.D. (biochemical oxygen demand) and the 15-minute I.D.O.D. (immediate oxygen demand) were carried out. The values of B.O.D. were less than 0.2 ppm and for the I.D.O.D. were negligible in all cases; they were therefore disregarded in the final oxygen consumption figures.

No appreciable change was noticed in the electrical conductivity of the water before and after the experimental run in the respiration chamber. This was measured with a type R.C. Conductivity Bridge (Industrial Instruments Inc., New Jersey, U.S.A.).

MARKING OF FISH

To recognize an individual so that it might be used for successive experiments, the fish were tagged by different coloured silk threads passing through their body just in front of the dorsal fin. A short dip in 1% salt solution was given after tagging. In most cases the fish were held for a period of at least 3 days before being used in an experiment. The few fish which showed abnormal behaviour or were suffering from infection at the end of this period were discarded.

EXPERIMENTAL RUN

The fish to be experimented upon was starved for a minimum period of 24 hours to avoid undue deposition of faeces in the respiration chamber during the period of experimentation.

Prior to the run, the chamber was placed in a water bath having the same temperature as that of the experimental run. Water charged with the desired concentration of oxygen and carbon dioxide was allowed to flush through the chamber, spilling over the top. A single fish was obtained from the acclimation tank with as little disturbance as possible and placed in the chamber as soon as it had filled with water. On an average a flow of 1200 cc/minute was used for the flushing and there was always sufficient to keep the difference of the oxygen concentration between the inflowing and outflowing water at 1 ppm or less. This preliminary period of flushing with the chamber stationary lasted for one hour after the introduction of the fish.

Since the experimental animal removed oxygen during the rest period, it was necessary to supply water with oxygen at slightly above the desired saturation at which the experiment was designed to be carried out in order to obtain the particular saturation desired at the

beginning of the actual experimental run. At the end of the hour samples were drawn from the chamber and set aside for analysis for dissolved oxygen, carbon dioxide, pH and alkalinity. The values obtained were designated as the initial values. The volume of water within the chamber was adjusted to 5000 cc and the respiration chamber with the fish was then placed on a turntable and was rotated at a speed of 20 revolutions per minute (rpm) with the stimulator activated. No flow of water through the chamber was maintained during the run.

The speed of rotation and the strength of shock were designed to elicit an approximation of the maximum continuous rate of oxygen consumption termed hereafter the *active* rate, and were based on a preliminary series of experiments to test for the best combination of rate of revolution and stimulus strength. Three individual speckled trout acclimated to 10°C were subjected to experimentation with initial oxygen at air saturation, using different speeds of rotation with or without electric stimulus. Five individual runs of each fish at each test with alternate 15-minute periods of rest and activity were repeated with one individual fish.

The data are summarised in Tables II and III. There is a highly significant difference

TABLE II. Mean values of O_2 uptake by 3 speckled trout, for different stimulations and for different revolution rates.

Items	Revolutions per minute				Mean O_2 consumption for different stimulations mg
	5 mg	10 mg	20 mg	30 mg	
No current	13.92	14.05	13.84	15.68	14.37
3 volts	25.38	22.38	23.10	25.92	24.20
6 volts	26.01	25.67	30.40	25.48	26.89
Mean O_2 consumption for different revolution rates, mg	21.77	20.70	22.45	22.36	

TABLE III. Summary of analysis of variance of the items in Table II.

Source of variation	Degrees of freedom	Sums of squares	Mean square
Total	59	1553.78	
Between revolutions	3	18.11	6.04
Between volts	2	840.61	420.32
Volts \times revolutions	6	42.87	7.15
Error	48	652.19	13.59

between the response of the fish under stimulation and without stimulation. The effect of rate of revolutions on the rate of oxygen uptake in the chamber is not significant. It was observed that at 30 rpm the fish could not maintain a steady rate of swimming and with 6 volts they moved erratically in the chamber, while with 20 rpm and 3 volts they were able to maintain a steady rate of swimming without any abnormal behaviour. Thus it was decided that 20 rpm and 3 volts were adequate to produce the active oxygen consumption. This standard was maintained for all further experiments.

The results of all further experiments were also based on 4 consecutive determinations of the active rate of oxygen consumption interspersed with alternate periods of rest for the fish. Four individuals were thus tested at each concentration and each temperature. Depending on the size of the fish, the fish were required to swim for a period of 15 to 20 minutes during each determination. Smaller fish were required to swim the longer period since their absolute rate of oxygen consumption was lower.

A jet of water having the appropriate temperature was sprayed on the outside of the chamber to keep the temperature steady during the period of the run. After the run, the chamber was stopped and samples for the determination of oxygen, carbon dioxide, pH and alkalinity were drawn as quickly as possible. The values obtained were designated as final values for an individual run. The difference between the initial and final value gave the measure of the oxygen consumption for the particular period of time.

The maximum time taken for these samplings after stoppage of the chamber never exceeded 2 minutes. On stopping the chamber, the first sample taken within 30 seconds was for dissolved oxygen, the other samples for carbon dioxide, pH and alkalinity followed in succession. Samples for dissolved oxygen were collected by siphon in 60-cc glass-stoppered bottles and fixed immediately after collection. At least 3 to 4 times the volume of the sampling bottle was displaced before the flow was stopped.

Samples for carbon dioxide were collected in a long narrow Nessler's tube and titrated as soon as possible; pH and alkalinity were measured as quickly as possible after collection and under no circumstances did the time elapsed between collection and measurement exceed 3 minutes.

When samples were drawn after the first run, the fish was allowed to rest for a period equal to the period that it had been stimulated. During this period, the chamber was again flushed with water carrying the desired concentrations of oxygen and carbon dioxide. The dissolved oxygen concentration of the outflowing water regained the original value of the beginning of the experiment within a period of 10 to 12 minutes. Four to 5 times the volume of the chamber was displaced during the period of rest. At the end of the period another set of "initial" samples were drawn for measurements of oxygen, carbon dioxide, pH and alkalinity. The same procedure for measurement of oxygen consumption was repeated.

Each experiment lasted from 3½ to 4 hours, and the 4 individuals were tested on the same day under the same conditions. On the completion of each experiment, the fish was returned to its respective acclimation tank.

For a given series, i.e. a particular oxygen concentration with different carbon dioxide concentrations, the same fish were used; a different carbon dioxide concentration being used on successive days. Between series at different initial oxygen levels, however, the fish were returned to the acclimation tank for a minimum period of one week. In a few cases experiments were performed with the same group of fish on the next day in water without carbon dioxide after they had been exposed to high carbon dioxide concentrations on a given day. These showed that the fish had completely recovered from the effect of carbon dioxide within 24 hours after transfer to normal waters. Thus there appears to be no cumulative effect of carbon dioxide when the same fish was used on successive days with different concentrations of carbon dioxide.

In a few cases, a fish which had been experimented upon in one set of conditions could not be used further because it had grown too large for the chamber. Under such circumstances, another fish was selected from the acclimation tank.

TABLE IV. Regression equations of $\log O_2$ consumption as mg/kg/hr (Y) plotted against carbon dioxide as ppm (x), with the initial oxygen at air saturation.

Species	Average weight	Temper-ature	Regression equation	Antilogarithm of intercept 'a'
	g	°C		mg/kg/hr
Speckled trout	164	10	$\hat{Y} = 2.5193 - 0.0075x$	334
" "	164	15	$\hat{Y} = 2.5870 - 0.0082x$	386
" "	164	20	$\hat{Y} = 2.5172 - 0.0048x$	329
Common sucker	33	20	$\hat{Y} = 2.4856 - 0.0030x$	306
Carp	35	20	$\hat{Y} = 2.9482 - 0.0033x$	888
"	61	30	$\hat{Y} = 2.9718 - 0.0025x$	937
Bullhead	115	10	$\hat{Y} = 2.1210 - 0.0032x$	132
"	90	20	$\hat{Y} = 2.4250 - 0.0034x$	266
"	102	30	$\hat{Y} = 2.5246 - 0.0024x$	335
Goldfish	72	10	$\hat{Y} = 2.4535 - 0.0044x$	284
"	52	20	$\hat{Y} = 2.5723 - 0.0020x$	373
"	74 ^a	30	$\hat{Y} = 2.7149 - 0.0026x$	528

^aAverage of 4 fish.

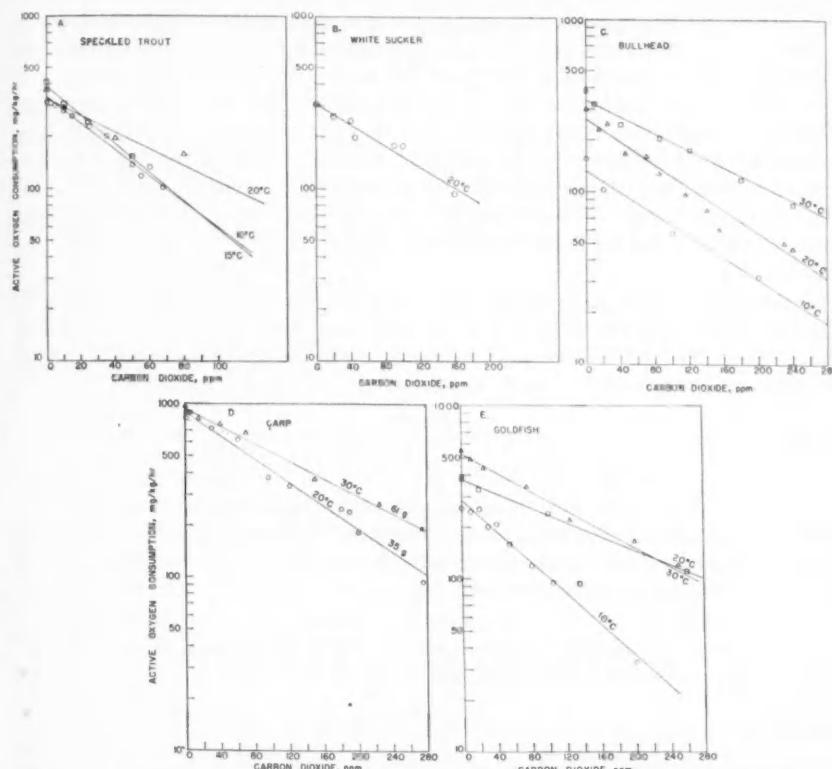


Fig. 3. Rates of active metabolism of 5 species of fish at various concentrations of carbon dioxide in the presence of initial oxygen at air saturation and measured at different temperatures. Each point is the average of 16 determinations and the lines of best fit were drawn according to the principle of least squares.

THE EFFECT OF DIFFERENT CONCENTRATIONS OF CARBON DIOXIDE, WITH OXYGEN AT AIR SATURATION, ON THE ACTIVE METABOLIC RATE AT VARIOUS TEMPERATURES

The active rates of oxygen consumption found for speckled trout, white sucker, brown bullhead, carp and goldfish, at an oxygen pressure of 160 mm with various levels of carbon dioxide, have been plotted on a semilogarithmic grid against temperature in Fig. 3. The semilogarithmic relation appears linear in all cases.

Straight lines have been fitted to the points by least squares taking oxygen consumption to be in "error." The equations of these lines are presented in Table IV.

SPECKLED TROUT

This species was tested at 10, 15 and 20°C. Tests were carried out at various concentrations of carbon dioxide up to a concentration of 75 ppm with the initial oxygen in all cases at air saturation (Fig. 3A).

As would be expected, the level of oxygen consumption changes with temperature at any given concentration of carbon dioxide. The temperature effect on levels will not be mentioned further in this section. Otherwise the results show that the active metabolic rate of this species is affected by the presence of carbon dioxide and diminishes proportionately with the increase in the carbon dioxide concentration in the medium. The proportionate rate of decrease is greatest at the two lowest acclimation temperatures. The temperature effect on decrease is significant, the "F" value being 5.98 with 2 and 13 degrees of freedom. The heterogeneity lies almost all in the difference between the slope of the line for 20° and those for 10 and 15°C.

Data for 3 individual fish at 10°C with different concentrations of carbon dioxide, in the presence of oxygen at air saturation, were subjected to analysis of covariance to find out if there was any significant difference in the effect of carbon dioxide between individuals. None was found ($F = 2.55$, d.f. 2 and 16).

WHITE SUCKER

Tests were carried out on this species only at 20°C. Carbon dioxide concentrations up to 160 ppm with initial oxygen at air saturation, were used for this purpose (Fig. 3B). Difficulties were experienced in keeping these fish steadily active in the respiration chamber even with electric stimulus up to 6 volts. Hence, this species was not used in further experiments.

Although it was difficult to make the fish continuously active, the results show the same proportionate depressant action of carbon dioxide on the oxygen consumption as was found for the other species.

BROWN BULLHEAD

Experiments were carried out at 10, 20 and 30°C, with carbon dioxide concentrations up to 250 ppm (Fig. 3C). The proportionate depressant effect of increasing carbon dioxide on the active metabolic rate was greatest at the two lowest acclimation temperatures. The temperature effect approached statistical significance ($F = 3.05$, d.f. 2 and 16).

Data obtained on 4 individual fish at 30°C with different concentrations of carbon dioxide, in the presence of oxygen at air saturation, were subjected to analysis of covariance to find out if there was any significant difference between individuals of the same group. None was found ($F = 2.42$, d.f. 18 and 3). However, as will be mentioned later, there was a significant heterogeneity in the response of groups of individuals at different times.

CARP

The effect of different concentrations of carbon dioxide up to 275 ppm with the initial oxygen at air saturation was tested with this species at 20 and 30°C (Fig. 3D). At both levels, again the depression of active oxygen consumption resulting from increased carbon dioxide was proportionate. The slopes of the semi-logarithmic lines exhibited the same trend with temperature, as mentioned for the

species above ($F = 17.05$, d.f. 1 and 13). Again no significant difference was found in the response of different individuals ($F = 1.08$, d.f. 3 and 21).

GOLDFISH

This species was tested at 10, 20 and 30°C. Tests were carried out at different concentrations of carbon dioxide up to a concentration of 250 ppm (Fig. 3E).

Good linear fits to the semilogarithmic relation were found for this species also and once more a change of slope with temperature was observed, the slope at 10°C being significantly greater than that found at 20 and 30° ($F = 32.5$, d.f. 2 and 15). The temperature responses at 30 and 20°C (Fig. 3E) were also significantly different ($F = 11.4$, d.f. 1 and 7). Data obtained on 4 individual fish at 10°C showed no significant difference in the effect of carbon dioxide between individuals ($F = 2.42$, d.f. 3 and 24).

THE EFFECT OF DIFFERENT CONCENTRATIONS OF OXYGEN AND CARBON DIOXIDE ON THE ACTIVE METABOLIC RATE AT A GIVEN TEMPERATURE

In a second series of experiments the temperature was held constant and the initial oxygen was varied as well as carbon dioxide. The data thus found have been plotted on a semilogarithmic grid in Fig. 4, and straight lines fitted by least squares as before. The equations for the lines of best fit are presented in Table V.

TABLE V. Regression equations of $\log O_2$ consumption as mg/kg/hr (Y), plotted against carbon dioxide as ppm (x), when the initial oxygen was varied.

Species	Av. weight	Tem- perature	Oxygen % air sat.	Regression equation	Antilogarithm of intercept 'a'
Speckled trout	g	°C	%		mg/kg/hr
" "	190	10	63	$\hat{Y} = 2.3660 - .0073x$	232.3
" "	190	10	44	$\hat{Y} = 2.2380 - .0061x$	172.9
" "	150	20	80	$\hat{Y} = 2.3719 - .0049x$	235.5
Bullhead	89	30	200	$\hat{Y} = 2.6060 - .0023x$	403.6
"	89	30	150	$\hat{Y} = 2.4320 - .0018x$	270.4
"	86	30	50	$\hat{Y} = 2.4840 - .0027x$	304.8
"	89	30	25	$\hat{Y} = 2.3820 - .0022x$	241.0
"	94	30	8.3	$\hat{Y} = 1.8200 - .0051x$	66.1
Carp	100	30	200	$\hat{Y} = 2.9652 - .0021x$	922.9
"	133	30	150	$\hat{Y} = 2.9343 - .0024x$	859.6
"	137	30	100	$\hat{Y} = 2.8569 - .0022x$	719.3
"	108	30	50	$\hat{Y} = 2.6996 - .0023x$	500.7
"	112	30	25	$\hat{Y} = 2.5041 - .0024x$	319.2
"	117	30	12.5	$\hat{Y} = 2.1478 - .0026x$	140.5
"	118	30	8.3	$\hat{Y} = 1.8072 - .0049x$	64.15
Goldfish	74	30	200	$\hat{Y} = 2.6850 - .0018x$	484.2
"	80	30	150	$\hat{Y} = 2.6740 - .0018x$	472.1
"	83	30	50	$\hat{Y} = 2.6350 - .0026x$	431.5
"	83	30	25	$\hat{Y} = 2.3700 - .0013x$	234.4
"	84	30	12.5	$\hat{Y} = 2.1460 - .0030x$	140.0
"	84	30	8.3	$\hat{Y} = 1.8210 - .0067x$	66.22

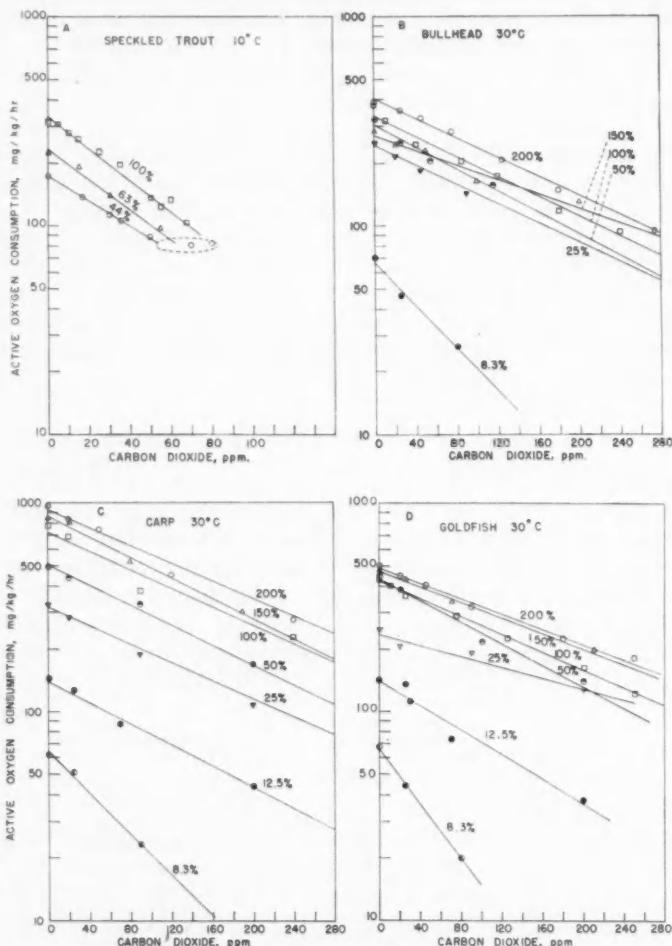


FIG. 4. Rates of active metabolism at various concentrations of oxygen and carbon dioxide for a given temperature. Percentage O₂ refers to percentage air saturation. The circumscribed area in Panel A shows where the speckled trout loses its ability to be active but maintains a more or less constant metabolic rate even with increase of CO₂. Each point is the average of 16 determinations, except the speckled trout and goldfish where they are the average of 12 determinations.

SPECKLED TROUT

This species was tested at 10°C at 100% air saturation (Table IV) and at two decreased levels of initial oxygen: 63% saturation and 44% air saturation (Fig. 4A). At each level of initial oxygen, the consumption of oxygen decreased linearly with increase in carbon dioxide, with one exception. At 44% saturation

of oxygen this linear relation did not entirely hold; instead, for speckled trout a minimum rate of oxygen uptake was encountered at 50 ppm CO_2 , which was maintained at 70 and 80 ppm CO_2 . At these levels of carbon dioxide complete loss of activity was observed. This departure from linearity was the only one in the whole series of experiments. No explanation can be offered at the present time for the divergence of these two points.

Because of the exception noted above, the slopes of the semilogarithmic lines for the speckled trout cannot be entirely satisfactorily compared. However, neglecting the two divergent points, the slopes of the lines for active metabolism in the presence of different concentrations of carbon dioxide at different oxygen levels show no significant difference ($F = 1.56$, d.f. 12 and 2).

BROWN BULLHEAD

Experiments were carried out at 30°C at 6 different levels of initial oxygen: 200%, 150%, 100%, 50%, 25%, and 8.3% air saturation (Table V, Fig. 4B).

The results show the same general trend of reduction in the active metabolic rate as the oxygen concentrations in the medium are decreased, with only one exception. At 150% air saturation lower values were found than those of the 100% and 50% air saturation series. A possible explanation might be that different groups of fish were used for the experiments at the different times. Accordingly experiments were repeated thrice at air saturation with the group of fish which had been tested at 150% air saturation. The fish still gave a lower value than the original group tested at air saturation and thus the discrepancy in the trend must be ascribed to differences among individuals.

A highly significant difference was found among slopes of the lines for active metabolism in the presence of different concentrations of carbon dioxide at different oxygen levels ($F = 8.01$, d.f. 5 and 18). The last group (8.3%) had a slope markedly different from the rest (Fig. 4B), the difference being highly significant ($F = 10.32$, d.f. 1 and 23) when compared with the remainder. No significant difference was found among the remaining groups ($F = 2.57$, d.f. 4 and 17). Thus it may be concluded that a significantly different response occurs only when the oxygen concentration is markedly reduced.

CARP

Seven initial levels of oxygen were tested at 30°C with various concentrations of carbon dioxide (Fig. 4C). The results once more show that the active metabolic rate is decreased in the presence of all levels of carbon dioxide as the oxygen concentrations are diminished in the medium. The response to increasing carbon dioxide is also again the same.

A significant difference in response to carbon dioxide at different levels of oxygen ($F = 3.97$, d.f. 6 and 14) was again found, and again it can be practically all ascribed to the difference in slope of the response of the lowest concentration of oxygen as compared to all the slopes at higher levels ($F = 10.88$, d.f. 1 and 20).

GOLDFISH

Experiments were carried out at 30°C at 7 levels of initial oxygen (Fig. 4D). The active metabolic rate was only slightly affected down to 50% air saturation. Below this the rate decreased as the oxygen concentrations were decreased in the medium. The semilogarithmic relation between oxygen consumption and carbon dioxide content was found to be linear in all the cases, and the slope of the semi-logarithmic line was greatest at the lowest oxygen level tested.

THE RELATION OF OXYGEN CONSUMPTION TO OXYGEN CONTENT OF THE MEDIUM

Prior to a discussion of the effects of carbon dioxide on metabolism, the effect of oxygen needs consideration. There are numerous observations on the effect of decreased oxygen on the rate of oxygen uptake. In general, reduced oxygen content below a certain minimum value, often referred to as the critical oxygen content, reduces the rate of oxygen uptake. This is particularly so if the fish are induced to be active (Fry, 1947; Fry and Hart, 1948; Graham, 1949; Job, 1955). The phenomenon is ordinarily termed respiratory dependence.

Certain data on the respiratory dependence of the species investigated here are already available in the literature. Comparisons can be made for three species, goldfish, bullhead, and speckled trout; no material for the carp appears to be available. Fig. 5 shows these values together with the present findings.

Two features are to be seen in Fig. 5. (a) The maximum steady rates of oxygen uptake which have been obtained by different authors vary greatly. In the case of the goldfish and the speckled trout, the rates determined here exceed the values reported in the literature. It seems probable that the higher rates reported in the present study were obtained because the improvement of the technique made it possible to stimulate the animal more positively. On the other hand the early values by Paul and Fry (MS, 1940) and Black, Fry and Scott (1939, and personal communication) for the bullhead considerably exceed the maximum values obtained in the present work, despite the fact that they were not obtained from fish stimulated electrically. This heterogeneity in the maximum effect of oxygen transport exhibited in the bullhead might indicate regional differences. The fish used by the workers cited above were from Algonquin Park, Ontario, whereas those used in the present study came from Lakes Ontario and Erie. (b) The rates observed by different authors converge in the lower region of respiratory dependence. From this it would appear that the range of respiratory dependence observed by the author who succeeded in obtaining the greatest active rate for a species is likely to exhibit the true range of dependence for that species, or at least the largest section of that range found to date.

In Fig. 5D, a comparison is made of the ability of the 4 species to take up oxygen from water at various oxygen levels. The highest rate of oxygen consumption found by any author has been taken for any given level of oxygen content. Three species can be compared at 30°C, the bullhead, carp and goldfish. The response of these 3 species is quite similar below an oxygen pressure of 80 mm Hg,

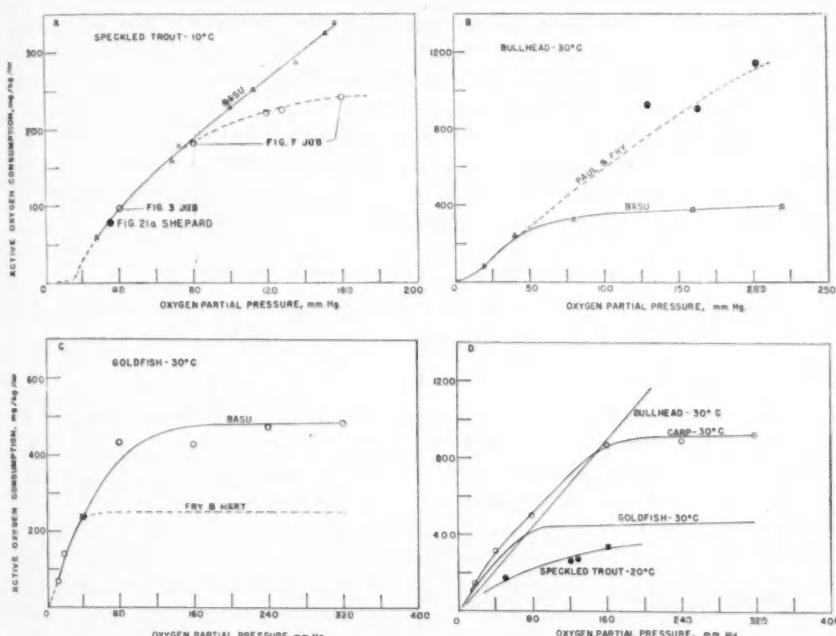


FIG. 5. Dependent active respiration of 4 species at various partial pressures of oxygen. In D a comparison of the respiratory dependence found here for the carp has been made with the other 3 species by taking the highest oxygen consumption values obtained by any investigator. Correction for weight of fish has been discussed in the text. The 20° curve for the speckled trout is taken from Fig. 10.

which is the region of dependence common to all of them. Above 80 mm Hg the goldfish becomes independent, and the curves diverge. The bullhead remains dependent up to the highest level of oxygen and has the highest rate. The carp is intermediate. The level at which the respiration becomes independent appears to be determined more by the ability to transport oxygen than any possible specific differences in the ability to extract oxygen from the environment. This was also shown for a series at 20°C by Fry (1957).

Data for speckled trout at 20°C are also shown in Fig. 5. A surprising feature is that this species shows the lowest absolute rate of oxygen consumption although the species are all probably at comparable places in their biokinetic range of temperature. A second feature is that the trout is less efficient in extracting oxygen from an environment low in that gas, although the comparison should really be made with the other species at 20°C.

THE RELATION OF TEMPERATURE TO OXYGEN CONSUMPTION

A second phenomenon that must be considered before we reach the discussion of the effect of carbon dioxide on respiration, is the effect of temperature. Further, when considering temperature, we need also to take into account the effect of the state of activity of the organism on the rate of oxygen consumption. Hence, two

levels of oxygen consumption will be considered: the rate when stimulated to continuous activity, which has been the main object of investigation in the present work—the *active* rate; and the rate under as nearly complete resting conditions as can be achieved, termed here the *standard* rate.

No measures of the standard rate were undertaken by the present author but estimates have been drawn from measurements available in the literature. All the measures of oxygen consumption dealt with in the present section refer to the case where the oxygen content of the water approximates air saturation.

ACTIVE RATES OF OXYGEN CONSUMPTION

The first problem is to compare the data obtained in the present investigation with the various data for the same species available in the literature (Fig. 6).

SPECKLED TROUT

Data on the active metabolic rate of this species have been presented by Graham (1949) and more extensively by Job (1955). Job carried out his determinations over a wide range of sizes (5 to 1,000 g) and thus has provided a basis for correction of his own and Graham's data to fish of the same size as those used in the present work. Job demonstrated that the relation $Y = a + bx$ held for his material, where Y is the logarithm of O_2 consumption, x is the logarithm of body weight, and b and a are constants appropriate to a given temperature. He gives values of "b" for 5, 10, 15, and 20°C. Thus by use of the equation:

$$Y_1 - Y_2 = b(x_1 - x_2)$$

various values of the metabolic rate can be corrected for a standard size of fish. The standard size chosen here is 164 g, which was the mean weight of the trout used by the present author.

The active rates of respiration found in the present work are practically identical with those found by Graham, and the values of all three authors correspond at 20°C. Job's values fall below the rest at 10 and 15°C. As suggested above, his fish were probably not stimulated to maximum effort. In consequence I feel satisfied that the present data represent a valid measure of the active metabolism of this species, and therefore that the drop in active metabolism at 20°C, found by Graham but not confirmed by Job, is real.

BULLHEAD

Data on the active metabolism of this species at various temperatures have been presented by Paul and Fry, in Fry (1947). The size of the fish used by them ranged from 12 to 20 g, average 15 g. The results have been adjusted to apply to 93 g, this being the average weight of the fish of the present determination, by using a slope of 0.8 for the value of "b". The data have been plotted (Fig. 6B) for comparison with the present investigation. The values obtained by Paul and Fry are consistently higher than the present determinations for the whole temperature range, the greatest difference being at 30°C. Moreover, in the present determinations it seems that at 30°C the temperature effect on active rate of oxygen consumption has reached a maximum and tends to drop off beyond that level, but in the case

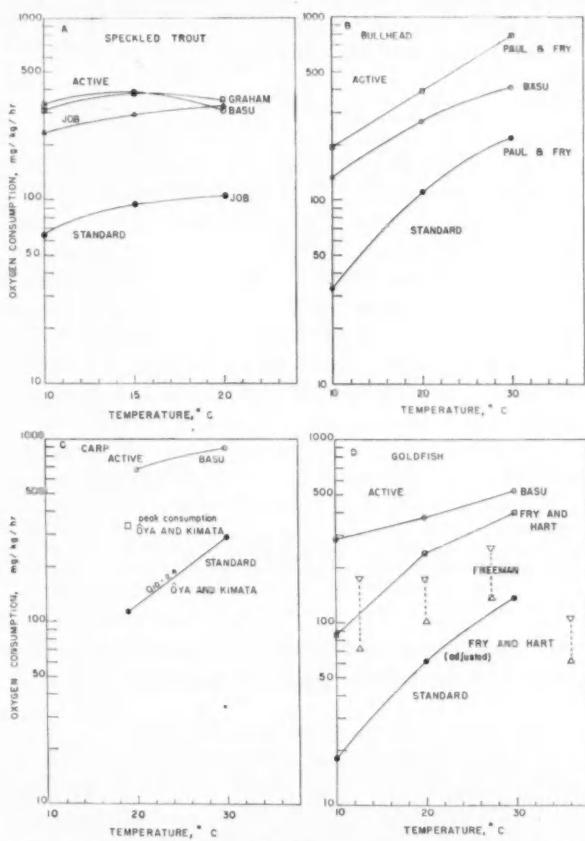


FIG. 6. Active and standard metabolism of thermally-acclimated fish. Correction for weight of fish has been discussed in the text. Oya and Kimata's data for 15-gram fish have been presented without correction for weight. Freeman's (1950) data are represented by the triangles.

of Paul and Fry's data, the oxygen consumption continues to show a steady rise. As shown by the above observations on the maximum rate of oxygen uptake by speckled trout and goldfish using the modified technique to stimulate fish to maximum effort, it seems quite unlikely that the present data would on that account yield a lower value than those of Paul and Fry, the determination of which was carried out using apparatus similar in principle to the unmodified activity chamber of Fry and Hart (1948).

As no other data are available at the present time to assess this discrepancy in the results, the only possible explanation that can be offered is the heterogeneity of this group of fishes, as discussed earlier in connection with the oxygen dependency curve. In the absence of further information to test this hypothesis of

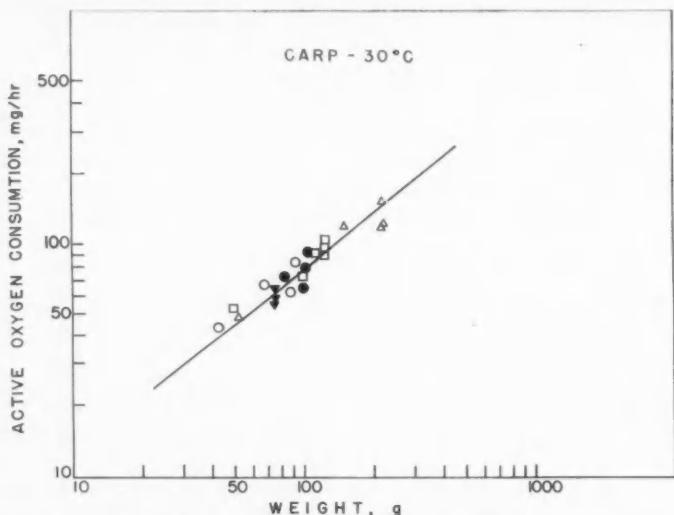


FIG. 7. The effect of size on the active metabolism of carp with initial oxygen at air saturation. The symbols designate individual marked fish. The slope of the calculated line is 0.8.

heterogeneity, the data of Paul and Fry (1947) should probably be taken as a valid measure of the active oxygen uptake in this species.

CARP

In an earlier series (Fig. 3C) the active metabolic rates in the presence of various concentrations of carbon dioxide when the initial oxygen was maintained at air saturation were measured for a group of fish, one acclimated at 20°C and the other at 30°C. The average size of the fish used at 20° was 35 g, and at 30° it was 65 g. In a later series (Fig. 4C), the active metabolic rates in the presence of various concentrations of oxygen and carbon dioxide were measured for a group of fish acclimated at 30°C. The average weight of the fish used was 104 g. To make the results comparable in Fig. 6C, the weights of the earlier series of fish have been corrected to a standard size of 104 g by using a slope of 0.8 obtained from Fig. 7.

The data in Fig. 6C show that with a rise of temperature from 20° to 30°C, there is an increase in the active metabolic rate. Unfortunately no observations were made at 10°C.

No data are available in the literature on the active metabolism of the carp for comparison with the present findings. The only supplementary information at all comparable with the measurements here is by Oya and Kimata (1938). They carried out experiments with carp at 19°C in a continuous flow system apparatus and observed a diurnal fluctuation in the amount of oxygen consumed by 3 fish (10–20g) over 24 hours. The peak point of their graph offers an approximate estimate of something less than the active rate and has been plotted in the graph

for comparison. It lies between the maximum rate as obtained in the present case and the standard rate obtained by them, a position that it could reasonably be expected to take.

GOLDFISH

Active rates as obtained by Fry and Hart (1948) for 4-gram fish at 10, 20 and 30°C have been plotted (Fig. 7D) to compare with the results for 74-gram fish obtained in the present investigation. Attempts were made to determine a mean slope for size correction of active metabolism from the data obtained in the present work but the range of size was too short to permit the estimation of a reliable value. Therefore, the data of Fry and Hart were plotted without correction for size of fish. However, Fig. 6 shows that the values obtained per unit weight are higher at each temperature tested than those of Fry and Hart (1948). Thus, unless the correction for size is an exception in this species from the apparently universal rule that the metabolic rate per unit weight remains constant or decreases with size, the present data reflect a higher state of stimulation than was obtained by Fry and Hart. The major difference which appears in the relative response of active oxygen consumption in relation to temperature is that the line obtained by Fry and Hart is steeper than that found in the present investigation. Fry and Hart's 10° value is quite low as compared to that of the present investigation, while that at 30° almost approaches the active rates determined here.

The most plausible explanation for such a difference is that it may be attributed to the different technique followed by the author to stimulate the fish to greater activity thus making it possible to obtain a higher oxygen uptake.

STANDARD RATES OF OXYGEN CONSUMPTION

In the present investigation, the minimum resting rate (i.e. the standard rate) of the different species was not measured and so estimates for this level have to be taken from the literature. Such estimates from the data of various authors are incorporated along with the active rates in Fig. 6. The details of this follow:

SPECKLED TROUT

Job's (1955) values for standard rates at different temperatures for a weight of 164 g were used directly.

GOLDFISH

Values for the standard rate of this species were obtained from Spoor (1946), Fry and Hart (1948) and Mookherjee (personal communication). Spoor obtained the value for a 32-gram male goldfish at an average temperature of 24°C. By extrapolation he provided the value of the standard rate at zero activity. Mookherjee carried out determinations on 3 fish (6.5–116g) at 22°C, using the same technique as was used by Spoor. Fry and Hart obtained values using 4-gram fish at temperatures from 5 to 35°C, taking the minimum value in a 24-hour cycle. From their data, a value of the standard metabolic rate at 22°C was obtained by interpolation. Further, Spoor's value at 24° was corrected to 22° by reducing it by an amount

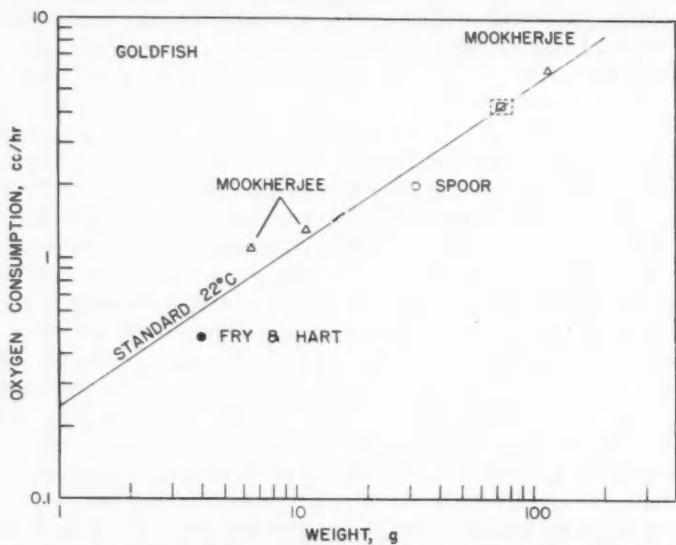


FIG. 8. The effect of size on the standard metabolism of the goldfish obtained by different authors, corrected to 22°C as indicated in the text. The equation for the line drawn is $Y = -0.6151 + 0.6698x$. The point outlined by the dashed line gives the value for a 74-gram fish used in calculating the standard rates at various temperatures.

required by the temperature coefficient found by Fry and Hart. The data of the three authors were then plotted in relation to size on a double log grid (Fig. 8). A regression line was calculated to give the best fit through these points. The value for the standard metabolic rate for 74-gram fish was then obtained from this line, its value being 4.3 cc/hr (83 mg/kg/hr). This point was taken as the anchor for the curve relating standard metabolism to temperature given in Fig. 6. Points at 20 and 30°C were estimated from temperature quotients based on the data of Fry and Hart as follows: 22 to 30°C, $Q_8 = 1.63$; 20 to 22°C, $Q_2 = 1.36$; 10 to 22°C, $Q_{12} = 4.88$.

BULLHEAD

The data of Paul and Fry for the bullhead were taken. A slope of 0.8 has been used for size correction from 5-gram to 93-gram fish.

CARP

Oya and Kimata (1938) carried out an investigation on this species at 19°C using fish with an average weight of 15 g, in which they observed a diurnal fluctuation in the metabolic rate. The minimum values shown on their curve have been chosen as an approximate estimate of standard rate. From the Q_{10} value of 2.4 given by Oya and Kimata, the proportionate rise in metabolic rate at 29° was estimated and a straight line was drawn joining two points now found for 19° and 29° and extrapolated to 30°C. No correction was made for size in these data.

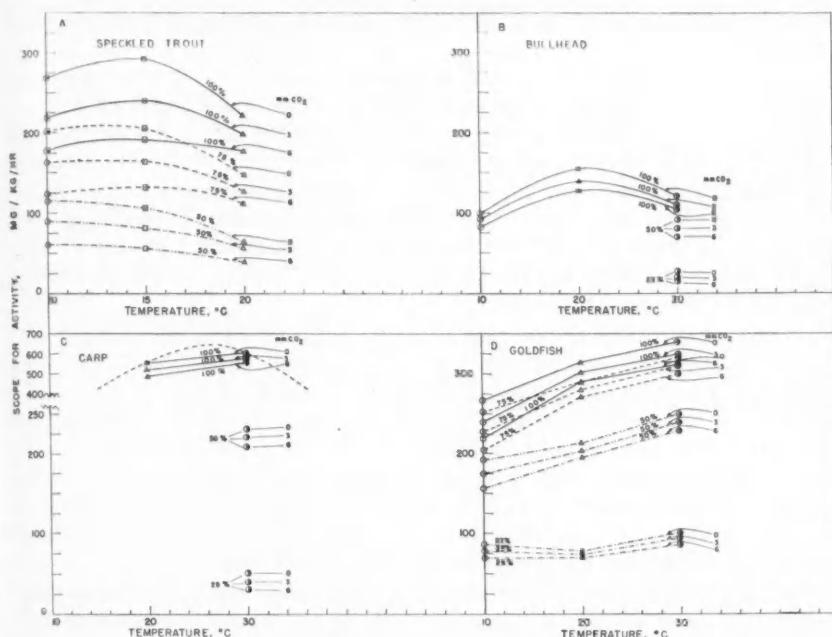


Fig. 9. Scope for activity of 4 different species in relation to temperature in the presence of various combinations of oxygen and carbon dioxide concentrations. The percentages of oxygen concentration are in terms of air saturation. In Panel C, the broken line indicates a probable scope value at different temperatures for the carp with oxygen at air saturation and no carbon dioxide.

THE RELATION OF TEMPERATURE AND CARBON DIOXIDE TO ACTIVITY

SCOPE FOR ACTIVITY IN RELATION TO TEMPERATURE WITH OXYGEN AT AIR SATURATION

The measure of metabolism that appears to be most indicative of the capacity of fish to be active is the difference between the active and standard rates. This has been termed the "scope for activity" (Fry, 1947). The scope for activity for the 4 different species investigated in the present case has been calculated from the data presented in Fig. 6. For example, in the speckled trout, at a weight of 164 g. and at 10°C, the active rate is 342 mg/kg/hr and the standard rate is 66. The scope for activity then is 276 mg/kg/hr. Similarly at 15 and 20°C the scopes are 291 and 220, respectively.

The data thus derived have been plotted in Fig. 9 as the topmost line in each panel. These topmost lines thus represent the scope for activity in water saturated with air both in respect to oxygen and carbon dioxide⁴ and thus provide the base

⁴The values as plotted are those calculated from the regression line with x (CO₂ ppm) equal to zero. The small correction for the content of free CO₂ in air-saturated water has been neglected.

line for discussion of the effect of carbon dioxide on activity in the sections which follow.

SPECKLED TROUT. When initial oxygen was maintained at air saturation in the absence of carbon dioxide, the greatest scope for activity was found at 15° , with an indication that the maximum scope for activity possibly lies in between 12° and 15°C (Fig. 9A). With the rise of temperature above 15°C , there was a gradual loss in scope; the maximum loss was at 20°C . These findings agree with the observations of Graham (1949), who found that the maximum scope obtained in speckled trout was at about 15° and fell sharply as the temperature rose higher. They apply only to fish weighing 174 g, since Job (1955) demonstrated a size effect in the relation of scope to temperature.

BULLHEAD. The scope for activity for the bullhead, calculated for fish of 93 g at 10, 20 and 30°C , with initial oxygen at air saturation in the absence of carbon dioxide, is presented as the topmost line in Fig. 9B. The data on bullhead pose a problem as the present findings do not agree with the observations of Paul and Fry (in Fry, 1947), who found a continuous rise in the active metabolic rate with increasing temperature until an upper incipient lethal temperature was reached which was approximately 35°C . The present data show that the maximum scope for activity is at 20° and falls off at 30° . Thus no definite conclusion can be reached until further evidence indicates the nature of this difference.

CARP. The scopes for activity for this species at 20° and 30°C are presented in Fig. 9C. Since the carp were tested at only two temperatures, the temperature-scope relation cannot be delineated, but scope rises with temperature up to 30°C . Although, actual data are not available, it seems by analogy with the other data presented in Fig. 9 that if a profile could have been drawn on the same basis, using 10°C data, the maximum scope for carp might be between 28 and 32°C . The suggestion that the fish would exhibit maximum scope between 28 and 32°C is somewhat supported by observations made by Pitt, Garside and Hepburn (1956), who found the final preferendum of carp to be 32°C —a temperature which all carp will eventually select regardless of their previous thermal experiences.

GOLDFISH. The scopes for activity at 10, 20 and 30°C , with initial oxygen at air saturation and no carbon dioxide are illustrated in the topmost line of Fig. 9D.

The scope of this species rises with rise of temperature up to 30°C . If we consider only the data of Fry and Hart (1948) for the same 3 temperatures we would see a curve very similar to that displayed here. However, their intermediate points at 15° and 25° , together with a further determination at 35° , show an optimum between 25° and 30° . This deduction from their data on metabolism was further supported by earlier observations made by Fry and Hart (1948) on the relation of temperature to cruising speed.

Unfortunately, I did not extend my observations beyond 30° , but am inclined to believe that the goldfish would have lost its scope for activity at about 35°C , as was observed by Fry and Hart, if the experiments had been continued. In any case the present observations at the temperatures investigated are in agreement with their findings although the scope found here is considerably greater.

SCOPE FOR ACTIVITY IN RELATION TO TEMPERATURE AND VARIOUS TENSIONS OF CARBON DIOXIDE WITH OXYGEN AT AIR SATURATION

Having discussed the effect of temperature on scope for activity, in the presence of initial oxygen at air saturation and without carbon dioxide, our next consideration is to find out what effect carbon dioxide has on scope for activity. The same procedure, i.e. subtraction of standard rates from active, was followed for calculating the scopes at various levels of carbon dioxide at different temperatures in the presence of initial oxygen at air saturation. The active rates of oxygen uptake at different levels of carbon dioxide were obtained from the regression equations in Table IV. The data for the 4 species with carbon dioxide at 3 and 6 mm Hg pressure⁵ are illustrated in Fig. 9.

The data show that for all species the scope for activity is decreased as the carbon dioxide concentrations in the medium are increased. In the case of the speckled trout (Fig. 9A) it is interesting to note that the scopes at the 3 different temperatures for 6 mm CO₂ are almost the same.

SCOPE FOR ACTIVITY IN RELATION TO TEMPERATURE AT VARIOUS LEVELS OF OXYGEN AND CARBON DIOXIDE

In the preceding sections we have discussed the effect of temperature on the scope for activity of the 4 species when the oxygen was maintained at air saturation, and when carbon dioxide was added to the medium. Our next consideration is therefore what happens when the oxygen concentration is reduced below air saturation, first without carbon dioxide, and secondly in the presence of carbon dioxide (Fig. 9).

Procedures for calculating the scopes at various levels of oxygen were the same as used for oxygen at air saturation, except that the values of the constant "a" at different levels of oxygen were derived from the smoothed oxygen dependence curves in Fig. 5, 10 and 11.⁶ The logarithms of the active "b" values used were those determined at the given saturation values when available i.e. at 30°C for the goldfish, bullhead and carp and 10°C for the speckled trout. At other temperatures, the slope associated with air saturation was used throughout since this was the only slope available.

For the speckled trout (e.g. see Graham, 1949) there is a significant loss in scope for activity even in the absence of carbon dioxide, when oxygen is reduced to 50% air saturation, although the addition of carbon dioxide in the medium lowers the scope farther. Thus effects of decreased oxygen are likely to be much more marked than effects due to any increase in carbon dioxide that would be possible in nature—and this is actually indicated in Fig. 9A.

Data for the bullhead at 30°C are shown in Fig. 9B as single points. The scope of the bullhead is not much decreased until the oxygen concentration is lowered to

⁵These levels of CO₂ cover the range ordinarily found in nature except under conditions of anaerobic metabolism.

⁶For reasons given below, the bullhead data are unsatisfactory; but it was felt, owing to the incomplete publication of the findings of Paul and Fry in Fry (1947), that it would be best to confine the discussion to the data obtained in the present work despite any uncertainty of its validity.

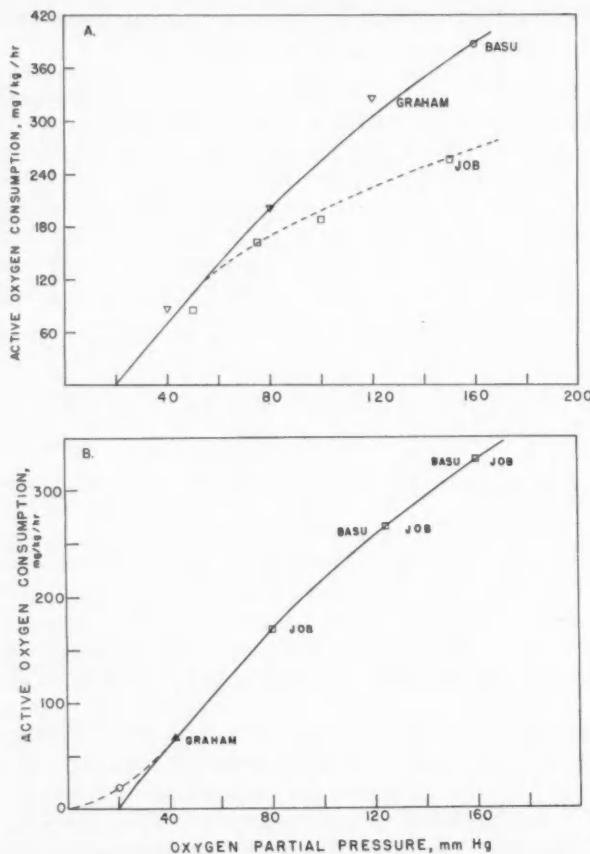


FIG. 10. Respiratory dependence of curves for the speckled trout at 15° (A) and 20° (B), obtained by Graham (1949), Job (1955), and the present author. The solid lines represent the maximum active rates obtained by different authors.

25% air saturation, while in the case of the speckled trout the scope is markedly decreased at 50% air saturation.

The effect of decreased oxygen on the scope for activity of the carp (Fig. 9C) appears to be of the same order as that for the goldfish discussed below.

The scope for activity of the goldfish (Fig. 9D) is the least affected of all fish at an oxygen pressure of 25% air saturation. At 50% air saturation it is affected similarly to that of the bullhead and the carp. The second feature which is observed at 25% air saturation oxygen is that the scope is diminished more rapidly at the higher temperatures so that the scope is essentially the same at all temperatures. This phenomenon is displayed by the speckled trout at 50% air saturation. At still lower levels of oxygen the scope would be expected to be less at higher than at

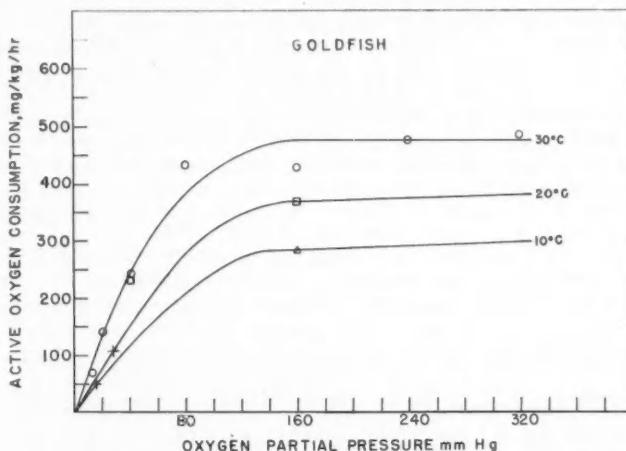


FIG. 11. The effect of temperature on the dependent active metabolism of the goldfish. The values are for fish weighing 74 g. Squares and crosses are calculations from the results of Fry and Hart (1948). The 10° and 20° lines were drawn through the points obtained by the author at 160 mm and Fry and Hart at 20 mm and otherwise made symmetrical with the 30° line.

lower temperatures (cf. Job, 1955)—a feature to be expected also in the speckled trout. The effect of carbon dioxide in diminishing the scope is slightly greater at low than at high temperatures at all levels of oxygen, as was noted in the speckled trout.

RESPIRATORY SENSITIVITY

If we choose some standard of reduction of the rate of oxygen uptake we can compare the sensitivity of various species by determining at what concentration of oxygen and carbon dioxide the respiration is reduced to such a standard. Four such standard combinations of oxygen and carbon dioxide will be considered in this section as a family of curves. These are, in descending order of magnitude:

1. Level of Half-Scope

The level at which the scope for activity of the animal is reduced to half the maximum scope.

2. Level of no Excess Activity

It was proposed by Fry (1947) that the oxygen level at which the rate of oxygen uptake is reduced to no more than the requirement for the standard metabolism can be taken as the minimum requirement for the existence of the organism. This he termed the level of no excess activity. Here the concept is extended to combinations of oxygen and carbon dioxide.

3. Asphyxial Level

The asphyxial level is determined by exposing samples of fish to a series of continuous flows of water carrying the desired concentrations of low oxygen over an extended period and noting the level which just brings about the death of the fish. The fish should not be stimulated to activity before or during the experiment. The asphyxial level will ordinarily be lower than the level of no excess activity since the standard level of metabolism ordinarily includes the cost of some physical activity (see Graham, 1949; Job, 1955).

4. Residual Level

This is defined as the oxygen left after the fish has died in a limited volume of water. Contrary to the procedure followed for the estimation of the asphyxial level in which a continuous flow system is used, the procedure followed is to seal the fish in a limited volume of water and to determine the oxygen present in it after the fish has died. Such procedure does not permit the replacement of oxygen in the medium as the oxygen concentration continuously diminishes through utilization by the fish. Thus the fish exerts its maximum capacity to extract oxygen from the medium until no further transport of oxygen to the cells is possible because of the stoppage of the heart. Therefore, a certain fraction of the transport continues while the fish is dying and the residual level may thus be considerably below the asphyxial level. The residual level is a somewhat indeterminate quantity since it will change to a certain extent with the relation of the size of the container to the size of the fish. But with the containers used by various workers, which have had volumes of the order of 10 to 50 times the volume of the fish, such changes have not been great and, as determined for a given species, the level can be taken as constant for all practical purposes.

To continue the thread of the discussion on the effect of various concentrations of oxygen and carbon dioxide on the various levels mentioned above, the level of half scope and the level of no excess activity will be derived directly from the respiration data presented above and the other two levels from the observations in the literature combined with the data on respiratory dependence.

The general principle applied in determining the various combinations of oxygen and carbon dioxide at which a given standard of respiration is met was to take the values in the regression equation for Y (log level of oxygen consumption relevant to the standard taken) and x , a given value of carbon dioxide, and then solve for " a " (using the appropriate slope value " b " for the species and temperature). This is in effect choosing one point on the regression line and extrapolating back to zero carbon dioxide to find the value of the oxygen required to maintain this standard of respiration. The level of oxygen concentration in the water, in the absence of carbon dioxide, which permits an active oxygen consumption of the value of " a " thus found is the level required in the water in the presence of the concentration of carbon dioxide taken to meet the standard in question.

The details of procedure followed to make these calculations in relation to the half-scope are as follows:

- (a) The values for the half-scope at different temperatures were obtained at air saturation, by reference to data illustrated in Fig. 9.
- (b) The standard rates at different temperatures were obtained from Fig. 6 and were added to the values obtained for the half-scope. This value was then taken as the rate of oxygen uptake when the scope was reduced to half. The logarithm of this value was substituted for the value of Y in the regression equation (Table IV).
- (c) The appropriate value of " b ", the slope, was obtained from experimental determination at any given temperature at air saturation.⁷
- (d) Several concentrations of carbon dioxide as parts per million CO_2 were chosen arbitrarily and their logarithms used for the value of x .

⁷The slope at air saturation was used in all cases as a convenient approximation although, as will be remembered, there were slight differences in the values found experimentally at different oxygen levels.

(e) Various values for "a", the intercept on the logarithmic axis, were determined by solving the equation. It will be remembered (page 192) that "a" is the logarithm of the rate of oxygen consumption in the absence of carbon dioxide.

(f) Having determined, from the antilogarithms of "a", the estimated oxygen consumption at zero carbon dioxide which will account for the half reduction in the scope in the presence of the various concentrations of carbon dioxide, the oxygen pressure which will permit this rate of consumption was obtained from the respiratory dependence curves (Fig. 5, 10, 11).

To determine the combinations of oxygen and carbon dioxide which suffice for the level of no excess activity the same procedure is followed. The only deviation is in the choice of the appropriate value of "Y" which is then the standard rate of oxygen uptake.

No direct determination of the rate of oxygen consumption is available for the asphyxial level. The observation is instead the level of oxygen content in

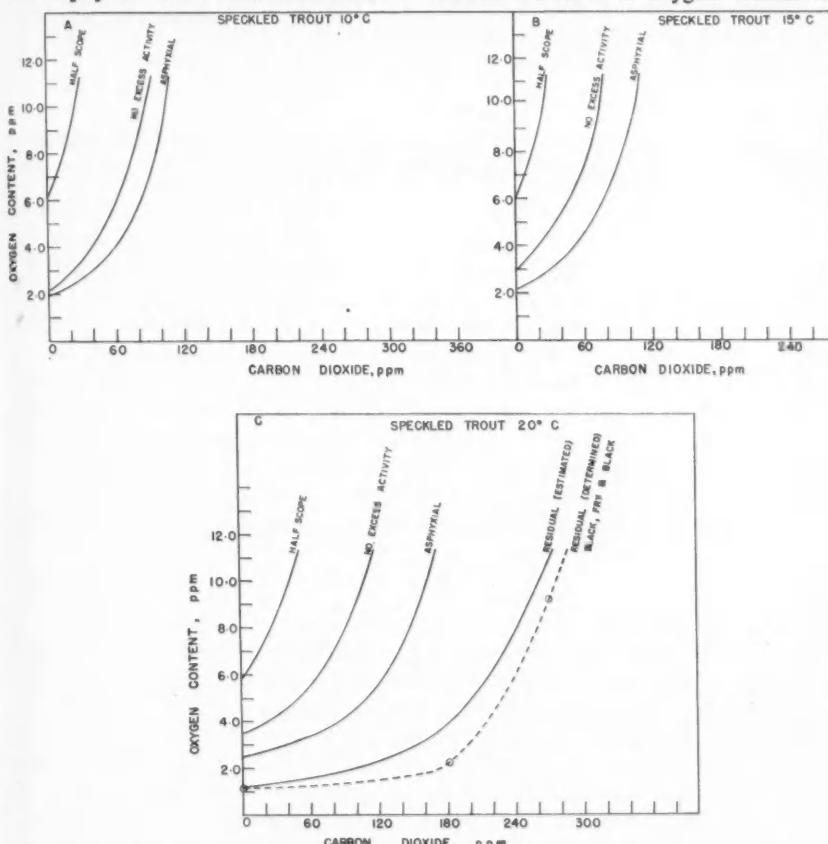


Fig. 12. Estimates of the influence of carbon dioxide on the utilization of oxygen by the speckled trout at different levels of activity. (The dotted lines are empirical determinations.)

flowing water in the absence of carbon dioxide which just kills half the sample (Shepard, 1955). Thus in contrast to the levels at half-scope and at no excess activity, the estimates of the oxygen consumption at the asphyxial level and the residual level are indirect measurements derived from the respiratory dependence curves by applying the observed level of oxygen under which the phenomenon was displayed. Otherwise the derivation of the points was the same in the two latter levels as outlined for the two former ones.

The combined effects of oxygen and carbon dioxide on the various levels under consideration are illustrated in Fig. 12, 13 and 15.

SPECKLED TROUT

The level of half-scope, the level of no excess activity, the asphyxial level, the estimated residual level and the determined residual level at various levels of oxygen and carbon dioxide at 10, 15, and 20°C are illustrated in Fig. 12. At 20°C, the determined data of the residual levels of oxygen in the presence of various concentrations of carbon dioxide obtained by Black, Fry and Black (1954) have been plotted as the lowest line for comparison.

BULLHEAD

Figure 13A gives the information. Except for the residual level (determined) by Hart (1957) between the temperatures 22–25°C, all levels illustrated are at

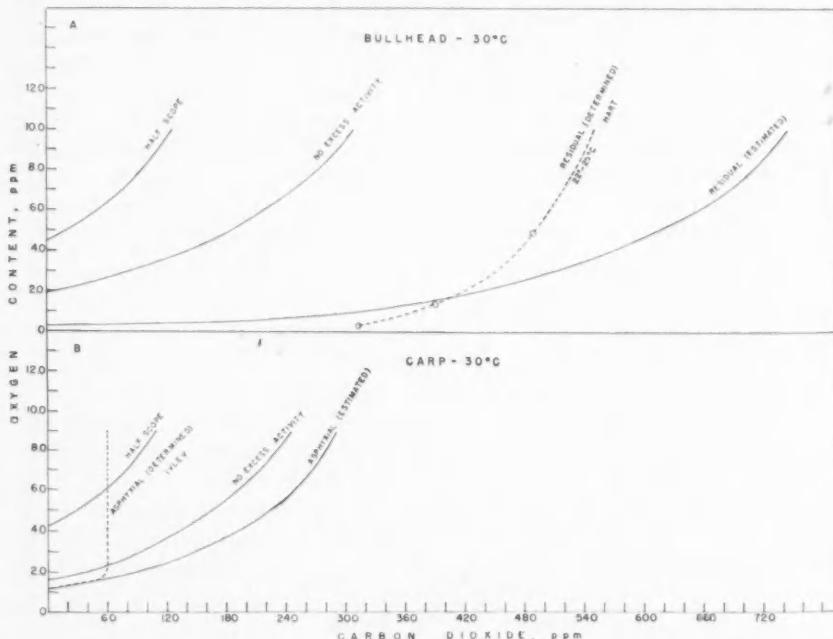


FIG. 13. Estimates of the influence of carbon dioxide on the utilization of oxygen by the bullhead and carp at different levels of activity. (The dotted lines are empirical determinations).

30°. Hart's data for 22-25° on the residual level were those closest to 30°C found for comparison. No temperature correction was made and the data of the residual curve of Hart are presented as such.

CARP

The respiratory sensitivity of the carp is illustrated in Fig. 13B. The empirical data of Ivlev (1938) for 30°C for asphyxiation at various levels of carbon dioxide have been presented. The asphyxial level obtained by Ivlev at zero carbon dioxide was 1.2 ppm O₂. The oxygen consumption at this level was found to be 200 mg/kg/hr from the respiratory dependence curve. This value has been used as the standard for calculating the curve for the estimated asphyxial level.

Ivlev's empirical data plotted in Fig. 13B do not agree with the theoretical curve calculated here, for he found the carp to be asphyxiated at all concentrations of oxygen at 50 ppm CO₂, while the asphyxial level estimated from the respiration data sweeps smoothly out to 250 ppm. No explanation for this disagreement can be offered at the present time.

GOLDFISH

The four standards of respiratory sensitivity for this species at 10°, 20° and 30°C are illustrated in Fig. 14. The estimates of the asphyxial levels are based on the data of Gardner, King and Powers (1922), together with the curves in Fig. 11. In the absence of any empirical data in the literature on the residual levels at 10°C at various levels of carbon dioxide, the empirical data of Fry, Black and Black (1947) at 7° and 15° have been plotted (Fig. 14A), with the assumption that data for 10° would occupy a position in between these two curves.

The empirical data of Fry, Black and Black (1947) at 20°C have been presented (Fig. 14B). It will be observed that the estimated residual level is slightly lower than the determined residual level beyond 360 ppm CO₂. No suitable explanation can be offered at this stage for this variation.

The residual levels at various concentrations of carbon dioxide determined by Fry, Black and Black (1947) at 25° and 32°C have been plotted in Fig. 14C as the closest curves in the absence of a 30° curve in the literature.

COMPARISON OF SPECIES

A single index may be obtained for each of the standards of respiratory sensitivity by finding the area to the left of the curve up to the air saturation value of oxygen. An index of this nature for the residual level was used by Fry, Black and Black (1947) and termed by them "the resistance to carbon dioxide". Hart (1945) proposed another single index, the partial pressure of carbon dioxide at which the residual level of oxygen was 80 mm Hg. He termed this index "the respiratory tolerance". The index of Fry and Black has been chosen here in preference to that of Hart. While it has been deemed proper to present the curves in terms of concentration of the dissolved gases in Fig. 12 and 14, it seems desirable, in order to partially compensate for temperature differences, to use the physical

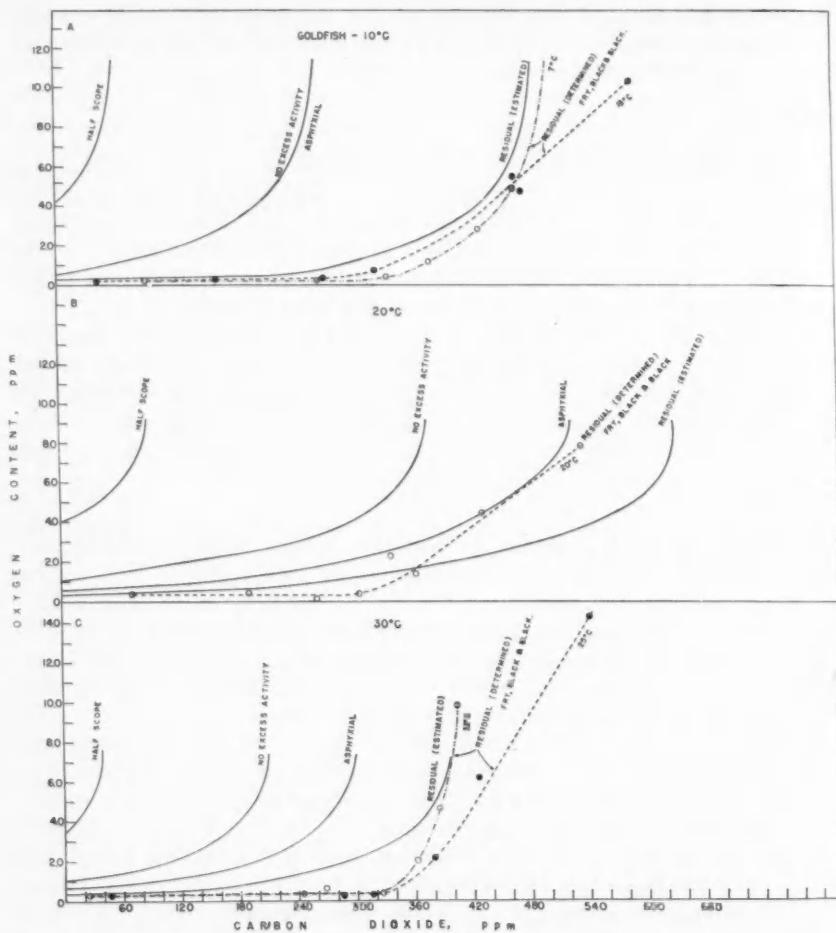


FIG. 14. Estimates of the influence of carbon dioxide on the utilization of oxygen by the goldfish at different levels of activity. (The dotted lines are empirical determinations.)

standard of partial pressure in the calculation of the index. The various indices are summarized in Table VI.

In these terms the goldfish is at least three times as resistant to combinations of low oxygen and high carbon dioxide as the speckled trout at 10° and 20°C. A comparison of the goldfish, carp and bullhead could be made only at 30°C. Here the carp is the most resistant of all to combinations of low oxygen and high carbon dioxide at all the levels chosen, the goldfish is least, and the bullhead is intermediate. At the level of no excess activity, the value for the goldfish is slightly higher than that for the bullhead.

TABLE VI. Comparison of the respiratory sensitivity of different species at different temperatures based on the area calculated for various indices. For details see explanation in text. The unit is (mm Hg oxygen) \times (mm Hg carbon dioxide).

Species	Temp.	Half-scope area	Level of no excess activity area	Asphyxial level area	Residual level area
	°C				
Speckled trout	10	399	2560	3092	9689
"	15	365	2036	3431	No data
"	20	514	2697	5606	11419
Goldfish	10	1334	10206	10206	21126
"	20	2544	17580	26274	35034
"	30	1442	12710	20548	28564
Carp	30	4490	20566	29533	No data
Bullhead	30	1972	11002	No data	47202

THE RESIDUAL RESPIRATION CURVE

Our next consideration is to discover the relation of the estimated residual curves based on the regression equations obtained in the present investigation with those of the residual curves determined experimentally by the several authors mentioned earlier. Our residual curves in general approach the residual curves determined in the literature.

Direct comparison could be made for the speckled trout and the goldfish. The greatest discrepancies occur at the point of inflection (Fig. 12, 14). They may be due to plasma transport. Anthony (personal communication) has demonstrated that goldfish can maintain resting metabolism, even in low concentrations of oxygen or in high concentrations of carbon dioxide, even though the erythrocytes are poisoned by carbon monoxide. Thus it is quite likely that in experiments in sealed containers in a limited volume of water, diminution of oxygen occurs so quickly that a significant fraction of the final oxygen is removed by plasma transport before the fish succumbs. If this were so the effect of increased carbon dioxide would be overestimated in the region of the lower end of the respiratory dependence curve. Under such circumstances the residual level determined would be lower than the residual level estimated from the values for the active oxygen transport.

THE COMPARATIVE SENSITIVITY OF FISH TO DIFFERENT PARTIAL PRESSURES OF OXYGEN AND CARBON DIOXIDE AS ILLUSTRATED BY THEIR BLOOD CHARACTERISTICS AND ACTIVE METABOLIC RATE

The blood even of the most sensitive species investigated here, the speckled trout, is at 20°C 95% saturated in a tonometer in the presence of 60 mm pO₂ (Irving, Black and Safford, 1941). On the other hand when the active respiration is considered, even the species with the least sensitive blood, the bullhead (Black 1940), has its active rate dependent on the oxygen content of the water in the absence of carbon dioxide at 160 mm pO₂ (Fig. 5). Thus in the case of the active rate of uptake of oxygen by fish, in the absence of carbon dioxide there is little

relation between the ability of the blood to take up oxygen and the rate at which oxygen is removed from the water. Apparently this is so because of the nature of the organs for respiratory exchange in fish.

Water and blood meet each other but once in a single counter-current passage through the gill, each on its respective side of the respiratory epithelium (van Dam, 1938). Thus the amount of oxygen taken up by the blood depends as much on the quantity of water coming in contact with the gill per unit time as it does on the partial pressure of oxygen in the water. The blood of lowest oxygen content meets the water of least oxygen content also. Hence a large fraction of the oxygen dissolved in the water can be taken up by the blood. The efficiency of the counter-current system in this respect was demonstrated by Hazelhoff and Evenhuis (1952). They showed in experiments with the tench that when the respiratory water flowed in the normal counter-current over the gills there was a mean utilization of 51% of the oxygen dissolved in the water, whereas when they reversed the direction of flow of the respiratory water the utilization fell to 9%. Because of the counter-flow of blood and water, the phenomenon of respiratory dependence can be best explained by considering it to be the result of the interaction between the capacity of the circulatory system to transport blood and that of the respiratory system to transport water. Over by far the greater range of environmental oxygen, the chemical characteristics of any fish blood allow it to be saturated with oxygen on leaving the gill, provided that the gill has been sufficiently irrigated during the passage of the blood through the respiratory lamella. However, even at air saturation, because of the difference in the oxygen capacities of the two media, blood and water, some 15 to 20 times the volume of water must pass through the gill per unit volume of blood. If the water were only 25% saturated, then 60 to 80 times the amount of water would have to pass per unit volume of blood to provide the necessary supply for its saturation.

Thus, although the partial pressure of oxygen in the water would still be sufficient to provide the gradient for complete saturation in most species (even in the speckled trout at 15°C), it is unlikely that the respiratory volume could be increased to provide the necessary mass of oxygen. In consequence there is little necessary relation between the oxygen transport properties of the blood and the phenomenon of respiratory dependence in the absence of carbon dioxide. It now remains to be seen what is the relation between the active uptake of oxygen and the effect of carbon dioxide on the dissociation curve.

The sensitivity of the bloods of four of the species investigated here, in terms of the effect of carbon dioxide on oxygen capacity, has been determined; the speckled trout by Irving, Black and Safford (1941) and Black (personal communication; see Fry, 1957), the carp, the common sucker and the bullhead by Black (1940). A direct comparison between the loading capacity of the blood and the rate of transport of oxygen by the active fish has been made in Fig. 15. There are some differences in temperature between the measurements on the blood and on the whole fish, but these differences however do not essentially affect the argument. The active respiration curves at 15°C for the sucker and the carp would be expected to have steeper slopes than the 20°C curves presented and thus lie below them.

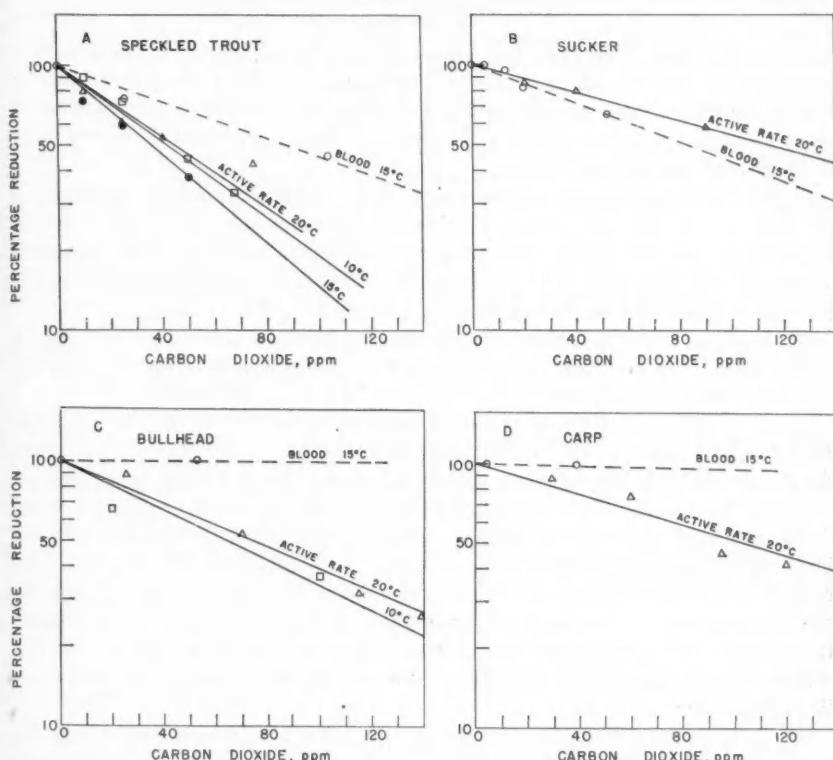


FIG. 15. Comparison of the effect of carbon dioxide on the oxygen capacity of the blood and the reduction in the rate of active respiration. All curves represent measures in the presence of oxygen at air saturation. Data for blood curves are from references given in the text.

The divergence between the blood curve and the active respiration curve varies greatly from species to species. It can be seen that the slopes of the two curves are almost identical in the sucker, diverge by a factor of about 2:1 in the speckled trout, 10:1 in the carp and almost infinity in the bullhead.

It may be argued that the disparity in response between the blood and the whole fish is the result of a spurious comparison, since the chemical transport of oxygen depends on the unloading capacity as well as the loading capacity of the blood. Unfortunately this circumstance cannot be examined at the present time since there are no data available for the oxygen content of venous blood of active fish. The suggestion is, however, from the data of Ferguson and Black (1941) for the speckled trout, that the oxygen content of the heart blood may approach zero under activity. All that can be said is that the relation between blood and active respiration is not the simple one to be inferred from the studies of fish blood that have been presented up to the present time.

SUMMARY

(1) Speckled trout, white sucker, brown bullhead, carp and goldfish were acclimated to various temperatures and then made to maintain a steady rate of activity by mild electric stimulus; their oxygen consumptions were measured in the presence of various combinations of oxygen and carbon dioxide at the temperature to which they had been acclimated.

(2) The rate of oxygen consumption in the presence of various combinations of dissolved oxygen and carbon dioxide can be expressed by a linear relation between the logarithm of the rate of oxygen consumption and the concentration of carbon dioxide for each level of oxygen saturation. A single exception to this general rule was found for the speckled trout (see page 188).

(3) The linear relation so found is characteristic of a given species at a given temperature. The sensitivity to carbon dioxide changes with temperature, being less at the higher temperatures.

(4) The oxygen concentration determines the active level of oxygen consumption in the absence of carbon dioxide, but does not influence the response to carbon dioxide except when the oxygen concentration approaches the lower lethal limit.

(5) The active rates of oxygen consumption experimentally determined here for the speckled trout and the goldfish, were compared with data available in the literature obtained under more or less similar conditions of activity. It was concluded that the present data were a satisfactory representation of the active metabolic rate for these species. The data for the bullhead were also compared with previous findings but the comparison led to no satisfactory conclusion concerning the validity of these data as representative of this species. The data for the sucker and the carp are new.

(6) The data found here for the active metabolism, for all species but the sucker, were combined with data for the resting (standard) metabolism available in the literature to produce estimates of the ability of these species to be active when exposed to various combinations of oxygen and carbon dioxide—called the scope for activity.

(7) The respiratory sensitivities of the 4 species were further compared by considering the estimated combinations of oxygen and carbon dioxide required to bring about asphyxiation in flowing water, and also with residual levels of oxygen and carbon dioxide observed when fish were confined in limited amounts of water. The rank of a given species with respect to sensitivity to carbon dioxide was the same by all the comparisons mentioned in items (6) and (7).

(8) Differences between empirical determinations of residual levels reported in the literature and the theoretical levels determined here can possibly be explained by the effects of plasma transport.

(9) The ability of the blood of a fish to take up oxygen in the presence of carbon dioxide shows no direct relation to the ability of the fish to transport oxygen from the external medium under activity.

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The Incidence of Nematodes in the Fillets of Small Cod from Lockeport, Nova Scotia, and the Southwestern Gulf of St. Lawrence¹

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ABSTRACT

The incidence of nematodes in fillets of Atlantic cod (*Gadus callarias*) was determined in 1957 in four areas within 10 miles of Lockeport, N.S., and in six areas in the southwestern Gulf of St. Lawrence. About 1,500 cod, mostly between 1 and 6 years of age, were examined. All nematodes examined (517) were larvae. About 97% belonged to the genus *Porrocaecum*; the remainder appeared to belong to the genus *Anisakis*. In all areas there was a progressive increase in incidence with increasing age of cod. In age-groups IV and V usually more than 70% of the cod were infected. Considerable local variation in incidence was observed in the Lockeport region. Cod were more heavily infected in inshore than in offshore waters. The samples from the Gulf of St. Lawrence showed less geographic variation in incidence than the Lockeport samples. The incidence in cod from the Magdalen Islands was noticeably lower than that in cod from the New Brunswick shore. Cod in the Gulf of St. Lawrence were infected to about the same extent as those from the offshore areas near Lockeport. The relation between local variations in incidence and the distribution of seals is briefly discussed.

INTRODUCTION

AN EARLIER PAPER (Scott and Martin, 1957) reported on the incidence of larval nematodes, mainly *Porrocaecum decipiens*, in fillets of cod of commercial size from fishing grounds off the southern Canadian mainland. In 1957, the incidence of these nematodes in small cod was determined in the vicinity of Lockeport, Nova Scotia, and in the southwestern Gulf of St. Lawrence. The cod in these areas were studied for quite different reasons. We were interested in determining the incidence of nematodes in the Lockeport area preparatory to the initiation of a program to reduce the numbers of harbour seals, *Phoca vitulina*, which are the definitive hosts of *P. decipiens* along southwestern Nova Scotia. In the Gulf of St. Lawrence we wished to compare the incidence in cod from the Magdalen Islands, where the harp seal, *Phoca groenlandica*, is abundant in the late winter and spring, with that in cod from the New Brunswick shore where harp seals are apparently much less common. It was hoped that such a comparison would clarify our understanding of the role of the harp seal as a source of *P. decipiens* for cod in the southwestern Gulf of St. Lawrence. For details on the distribution of seals and the incidence of *P. decipiens* in seals along the east coast, the reader is referred to papers by Fisher (1950) and by Scott and Fisher (1958). Although the immediate objects of these two studies were unrelated, the results from each bear on the relationship between seals and the infection in cod.

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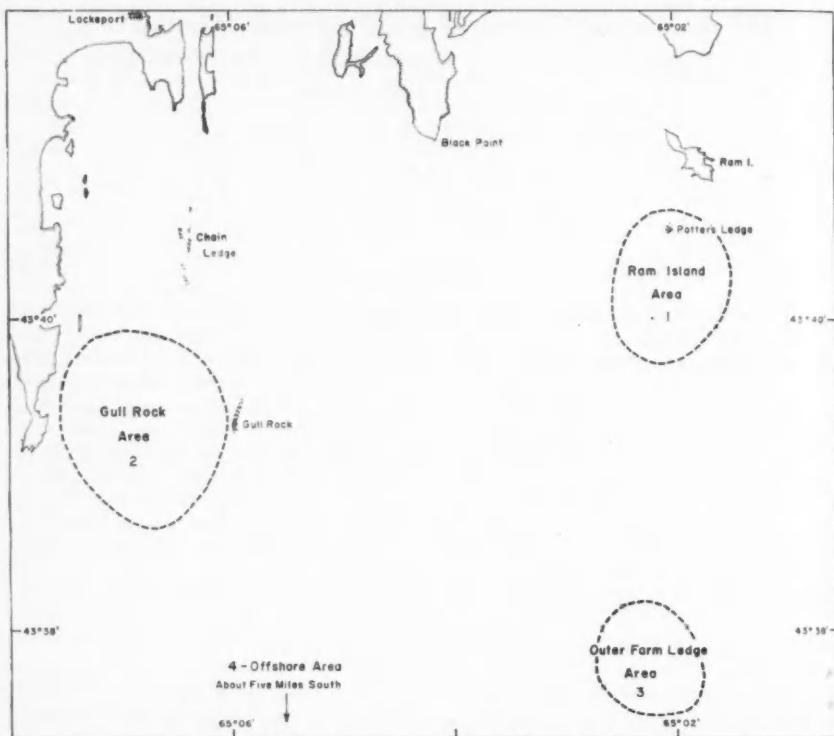


FIG. 1. Map of the area around Lockeport, N.S., showing places mentioned in the text.

METHODS

About 950 cod, from four areas near Lockeport, N.S., were examined for nematodes in two periods: May 17 to June 25 and August 17 to September 6. These cod were mainly taken from three inshore shoal areas (less than 15 fathoms deep): Ram Island, Gull Rock and Outer Farm Ledge (Fig. 1). Eighty-five cod were caught in deeper water (about 30 fm deep) 10 miles south of Lockeport. This group is referred to as the Offshore sample. In June, with a few exceptions, no cod longer than 60 cm was examined. In August, no cod longer than 55 cm was examined.

Samples of about 100 cod, each less than 60 cm in length, were taken from each of six areas in the Gulf of St. Lawrence during the period July 25 to August 9: one each from near Entry Island (16 fm) and near Brion Island (20 fm) in the Magdalen Islands, and one each from four localities along the New Brunswick shore, namely, Miscou Point (18-20 fm), southeast of Shippagan Island (38-42 fm), mouth of Miramichi Bay (12 fm), and south of Point Escuminac (11-15 fm). More precise locations of these fishing areas are indicated in Fig. 2 and in Table I.

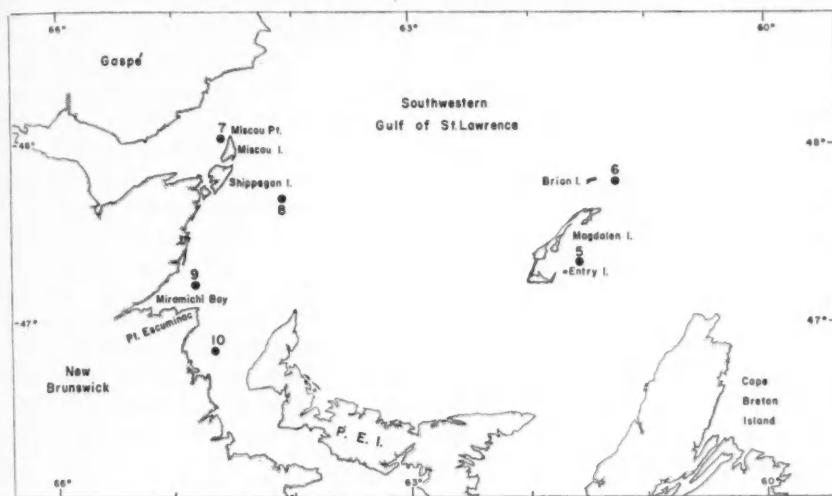


FIG. 2. Map of the southwestern Gulf of St. Lawrence showing places mentioned in the text.

For each cod, we recorded for length to the nearest centimetre, age as determined by examination of otoliths, and the number of nematodes in the fillets.

Most of the cod taken near Lockeport were examined by Scott. In August and September, Messrs C. J. Bayers and M. F. Fraser assisted with the examination of cod. Analysis of the records of the three observers indicated no personal bias so all the data have been grouped. No consistent seasonal differences were noted in the Lockeport samples. Accordingly, the samples collected in May and June and in August and September have been united.

C. J. Bayers examined all the cod in the samples from the Gulf of St. Lawrence.

Determinations of age were made by Mr G. W. Condon for the Lockeport samples and by Mr P. M. Powles for the Gulf of St. Lawrence samples.

The following procedure for the determination of the number of nematodes in a cod was consistently observed. Each cod was gilled, gutted and thoroughly washed. In filleting, the anterior vertical cut was made as close as possible to the posterior margin of the bases of the pectoral and pelvic fins. This cut was extended forwards to the hind edge of the skull. Flesh adhering to the vertebral column or to the ribs was examined for nematodes. No attempt was made to find nematodes which may have been present in the residual flesh attached to the head. Each fillet was skinned. The inner surface of the skin was examined for nematodes before the skin was discarded. Then one of the pair of fillets was inspected superficially for conspicuous nematodes which, if present, were removed and counted. The fillet was then cut transversely into $\frac{1}{4}$ -inch slices, each of which was examined for nematodes. This procedure was repeated for the other fillet of the pair. The total number of nematodes in both fillets was recorded.

IDENTITY OF THE NEMATODES

In June, 401 nematodes and in August, 116 nematodes were collected by Scott as each appeared in fillets being examined by him at Lockeport. All were collected from cod caught in the areas of Ram Island, Gull Rock and Outer Farm Ledge. Cut or broken nematodes were not collected. With those exceptions, the samples of nematodes should be representative of the population of nematodes in inshore cod at Lockeport at the time of sampling. The nematodes were killed in hot 70% ethyl alcohol and preserved in fresh 70% glycerin-alcohol. Each nematode was subsequently dissected under a binocular microscope. All were larvae. Nematodes belonging to the genus *Porrocaecum* made up 98% of the sample in June and 95% in August. The remainder were tentatively identified as of the genus *Anisakis*.

No identifications were made of nematodes from cod from the Gulf of St. Lawrence. Templeman, Squires, and Fleming (1957) stated that of 427 nematodes examined by them from cod from the southwestern Gulf of St. Lawrence all were larval *Porrocaecum*. On this basis, therefore, most nematodes found in this region by us were *Porrocaecum*.

OBSERVATIONS AT LOCKEPORT

The incidence of nematodes in cod from the Lockeport region varied greatly in the different samples (Table I). Only those samples with 10 or more cod with one exception have been considered in Table I. This variation was obviously related to two main factors: age of cod and locality.

There was a progressive increase in infection with increasing age of cod in the four areas studied at Lockeport. The incidence of nematodes was lowest in age-group I. Only three cod in this group of 21 small cod from all four areas were infected. The smallest infected cod was 13 cm long. In successively older age-groups the incidence usually increased with each age-group until more than 70% of the cod in most age-groups IV and V were infected. In the inshore areas of Ram Island, Gull Rock and Outer Farm Ledge there was approximately a two-fold or threefold annual increase in the mean number of nematodes in age-groups II to IV. On the other hand, the samples from the Offshore area showed a much lower rate of increase between age-group II and IV. The incidence in age-group V from Outer Farm Ledge and the Offshore area was lower than that in age-group IV from the respective areas. This lower incidence may have resulted from our arbitrary selection of cod less than 60 cm long. By so doing, we excluded the fastest growing cod in age-group V. We have evidence that the fastest growing cod in an age-group were more than twice as heavily infected as the slowest growing group. Accordingly, our selection of cod on the basis of length probably excluded many of the most heavily infected cod in age-group V. As a result, the observed incidence of nematodes in this age-group was probably appreciably lower than the real incidence.

Despite the fact that the two most widely separated sampling stations, Ram Island and Offshore, were only about 8 miles apart, considerable local variation in incidence was observed. In age-group II the mean number of nematodes per

TABLE I. Incidence of nematodes and mean lengths (cm) of cod in successive age-groups of cod from four areas near Lockeport, N.S., and from six areas in the southern Gulf of St. Lawrence in 1957. The range in numbers of nematodes in the central 75% of each sample of cod is given.

Area	Age-group	No. of Cod	Mean length	Mean no. nematodes per cod	Incidence	75% range
<i>cm</i>						
LOCKEPORT						
1. Ram Island	II	60	37	1.4	63	0-3
	III	140	44	4.4	71	0-9
	IV	46	51	8.0	78	0-22
2. Gull Rock	II	116	34	1.3	48	0-3
	III	123	44	2.9	77	0-6
	IV	40	49	8.7	73	0-9
3. Outer Farm Ledge	II	38	33	0.34	29	0-1
	III	112	41	1.6	56	0-3
	IV	72	49	2.9	79	0-6
	V	34	55	1.7	77	0-3
4. Offshore	I	21	13	0.14	10	0
	II	6	29	0.67	67	0-1
	III	21	39	1.1	52	0-3
	IV	17	46	1.3	71	0-3
	V	10	53	1.0	50	0-3
<i>GULF OF ST. LAWRENCE</i>						
5. Entry Island 47°19' N., 61°34' W. 16 fm	II	28	29	0.14	14	0
	III	23	35	0.22	22	0-1
	IV	32	48	1.3	59	0-3
	V	12	56	1.7	67	0-2
6. Brion Island 47°47' N., 61°16' W. 20 fm	II	16	31	0.24	19	0-2
	III	31	36	0.32	23	0-1
	IV	26	47	0.81	46	0-2
	V	15	53	0.80	40	0-2
7. Miscou Point 48°02' N., 64°36' W. 19 fm	II	20	28	0.20	20	0-1
	III	26	35	0.50	35	0-1
	IV	31	47	1.6	77	0-4
	V	14	53	2.4	79	0-5
8. Shippagan Island 47°42' N., 64°05' W. 40 fm	II	11	29	0.64	36	0-2
	III	35	37	0.91	66	0-2
	IV	32	47	1.3	66	0-3
	V	17	54	4.1	94	2-7
9. Miramichi Bay 47°12' N., 64°49' W. 12 fm	II	16	28	0.19	19	0-1
	III	23	37	0.65	39	0-2
	IV	33	46	1.5	79	0-3
	V	16	54	2.5	88	1-4
10. Point Escuminac 46°50' N., 64°40' W. 15 fm	II	11	30	0.18	18	0-1
	III	31	37	0.68	45	0-2
	IV	38	48	1.9	84	0-3
	V	17	54	3.6	88	1-7

cod ranged from a minimum of 0.34 at Outer Farm Ledge to a maximum of 1.4 at Ram Island. In age-group III the values ranged between 1.1 nematodes per cod in the Offshore sample and 4.4 nematodes at Ram Island. In age-group IV the minimum and maximum values of 1.3 and 8.7 nematodes were recorded, respectively, in the Offshore and Gull Rock samples. In other words, the cod at the

farthest inshore stations of Ram Island and Gull Rock were much more heavily infected than those taken from Outer Farm Ledge or Offshore. Similar qualitative differences between the areas may be noted in the percentage incidence and also in the 75% range. These differences between the areas of highest and lowest infections are further emphasized by noting that the values recorded for age-group II cod from Ram Island and Gull Rock are very similar to those recorded for age-group III fish from Outer Farm Ledge and Offshore.

The frequency distributions of the numbers of nematodes in individual cod in the various samples were compared using the chi-square test of homogeneity. The probabilities that the observed differences between the distribution were attributable to chance are shown in Table II. We conclude that for age-groups II and III Ram

TABLE II. The probability (P) that differences between the frequency-distributions of the number of nematodes per cod in pairs of samples from four areas were due to chance is shown.

Age-group II		P
Ram Island	vs Gull Rock	.05-10
Ram Island	vs Outer Farm Ledge	<.01
Gull Rock	vs Outer Farm Ledge	<.05
Age-group III		
Ram Island	vs Gull Rock	.02
Ram Island	vs Outer Farm Ledge	<.01
Ram Island	vs Offshore	<.05
Gull Rock	vs Outer Farm Ledge	.02
Gull Rock	vs Offshore	0.5-10
Outer Farm Ledge	vs Offshore	.90
Age-group IV		
Ram Island	vs Gull Rock	0.15
Ram Island	vs Outer Farm Ledge	0.4
Ram Island	vs Offshore	.05-10
Gull Rock	vs Outer Farm Ledge	0.5
Gull Rock	vs Offshore	0.20
Outer Farm Ledge	vs Offshore	0.15

Island was the most heavily infected area, followed in turn by Gull Rock, Outer Farm Ledge and the Offshore areas. In older age-groups the same sequence existed but the differences were not statistically significant. This suggests that cod of the younger age-groups were more sedentary than older fish. The local differences further indicate the presence of stocks of cod which do not freely intermingle. This inference is supported by the fact that in each of age-groups II, III and IV the Ram Island cod were the longest and the cod from Outer Farm Ledge were the shortest. Furthermore, there was a greater preponderance of young cod in the Ram Island and Gull Rock samples than in the Outer Farm Ledge. Because of differences in the gear used to capture the Offshore fish, the Offshore sample cannot be accurately compared with the others. However, it appears that Offshore cod were slower-growing. Differences in coloration of the cod in the various samples were consistent with the idea that different stocks of cod were present. All cod taken Offshore had grey-green backs and white bellies. More than 50% of the cod at the three inshore stations had a marked reddish or brownish coloration.

Seals were not abundant in the Lockeport area. Despite the presence of many reefs and ledges apparently suitable for use by seals as hauling-out spots, the Ram Island area, particularly around Potter's Ledge and Black Point, seemed to be the only area of concentration of seals. Here, four or five seals were seen on two occasions. Elsewhere only single seals were seen. We doubt that the total seal population during the summer of 1957 within a 5-mile radius of Lockeport exceeded 50.

OBSERVATIONS IN THE SOUTHWESTERN GULF OF ST. LAWRENCE

The incidence of nematodes in the samples of cod from six localities in the southwestern Gulf of St. Lawrence increased with increasing age of cod (Table I). The mean number of nematodes per cod in age-group II was usually much less than one and the percentage incidence was generally less than 20. In cod of age-group V, the mean number of nematodes was as much as 20 times higher. Percentage incidences were also much higher in this age-group, being over 75% in four of the six samples.

Some geographic variation in the incidence of infection may be noted. The values for the two samples from the vicinity of the Magdalen Islands were lower than those in comparable age-groups in the other samples. Using the chi-square test of homogeneity we compared the frequencies of the numbers of nematodes observed in individual cod from three areas. These distributions were derived from the combined data of two samples from each area: Magdalen Islands (Entry Island and Brion Island), northern New Brunswick (Miscou Point and Shippegan Island), and Miramichi Estuary (Miramichi Bay and Point Escuminac). No significant differences (values of P all greater than 0.05) were found between the samples of cod of age-group II. Miramichi Estuary samples of age-group III, IV and V and age-groups III and V, but not IV, from northern New Brunswick differed significantly (P values $< .03$ in all cases) from the samples of cod from the Magdalen Islands. No difference at the 5% level of probability was observed between the Miramichi Estuary and the northern New Brunswick samples. We conclude that cod from the New Brunswick shore were more heavily infected with nematodes than cod from the Magdalen Islands.

No geographic variation was observed in the mean lengths of the cod of different age-groups from the six localities. Indeed, considering the huge area and the small samples involved, there was a remarkable similarity in the rates of growth in the different samples.

The mean number of nematodes per cod, the percentage incidence of infection and the variation in the number of nematodes in individual cod (as indicated by the 75% range) were usually much less in the Gulf samples than in the three in-shore samples from Lockeport. Infection levels in the Gulf samples, except in age-group V, were similar to those observed in the Offshore sample at Lockeport.

DISCUSSION AND CONCLUSIONS

Our observations show that cod may be infected by the time they are one year old and have reached a length of 13 cm. Similar observations have been made on cod from Passamaquoddy Bay, N.B., and the Bras d'Or Lakes, Cape Breton

Island. The presence of nematodes in cod of this age and size is obviously widespread. Although it is not certainly known how cod pick up these nematodes, there is some evidence that another animal serves as a vector for small nematodes before they enter cod. If so, this vector is probably an invertebrate since the diet of small cod in the southwestern Gulf of St. Lawrence and in the Bras d'Or Lakes consists almost entirely of invertebrates (Powles, 1958; W. F. Black, personal communication).

Cod in the inshore Lockeport area were clearly more heavily infected than cod a few miles offshore. This difference can be attributed to at least two causes. First, harbour seals which are the ultimate source of infection in this region confine themselves very closely to the shoreline. By so doing, the dispersal of the parasite tends to be restricted to inshore waters. Secondly, judging by differences in the rate of growth and by differences in the coloration of the fish, the inshore cod as typified by those around Gull Rock and Ram Island form a population distinct from an Offshore population. Populations in this area are essentially sedentary (McCracken, 1956). Accordingly, the resident inshore cod will have a much greater opportunity to become infected. These stocks do mix to a certain extent, as shown by the intermediate nature of the values observed in cod from Outer Farm Ledge which is between the two most inshore stations and the Offshore station.

In the Lockeport area a few harbour seals are capable of maintaining high levels of nematode infection in inshore cod in the same area. The influence of seals rapidly decreases as we pass from inshore to offshore areas. Nevertheless, even in the Offshore area at Lockeport, most of the older cod were infected. This conclusion that a few seals may support a high incidence of infection in sedentary populations agrees with observations made in the Bras d'Or Lakes on the relation between seals and infected cod (Scott and Fisher, 1958; Scott and Black, unpublished data).

There was much less local variation in the incidence of nematodes in cod from the Gulf of St. Lawrence than was observed in the Lockeport area. This and the similarity in the rates of growth of the cod in the various samples suggest that cod in the southwestern Gulf are not sedentary. This suggestion is consistent with the observation, based on tagging studies by McKenzie (1956), that there are extensive movements of cod of commercial size in and out of the Gulf of St. Lawrence. The incidence of nematodes was significantly higher in cod from the New Brunswick shore than in cod from the Magdalen Islands. This indicates that the stocks of cod in these areas do not freely intermingle. A similar suggestion has been advanced by McCracken (personal communication, 1958) on the basis of the recapture of cod tagged along the New Brunswick shore.

In the absence of detailed knowledge of the distribution and abundance of seals in the southwestern Gulf, it is difficult to relate with assurance the incidence of nematodes in cod to the presence of a particular species of seal. It is known, however, that the Magdalen Islands lie close to an enormous concentration of harp seals which are in the southwestern Gulf between January and June. It is also known that harbour and grey seals, which are much less abundant than harp seals, occur around the Magdalen Islands and, perhaps more commonly, along the New

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Brunswick shore between the Miramichi Estuary and Shippegan Island. Scott and Fisher (1958) have shown that harbour and grey seals are individually much more heavily infected with adult *P. decipiens* than harp seals. They further showed that the total number of adult *P. decipiens* in the population of harp seals was probably not greater than that in the combined populations of harbour and grey seals. If the harp seal is the most important source of these nematodes in Gulf cod, then we would expect that cod from the Magdalen Islands would be more heavily infected than cod from the shore of New Brunswick. Instead, the reverse was true. In the Lockeport area a few harbour seals supported an infection in Offshore fish as high as that observed in comparable age-groups of Gulf cod. On the basis of numbers alone, there are sufficient grey and harbour seals in the southwestern Gulf to account for the levels of infection observed there. Furthermore, many Gulf cod spend much time outside the Gulf and probably acquire part of their infection in areas remote from any possible influence of the harp seal. We must conclude, therefore, that there is no evidence from the present study that the harp seal is the principal source of *P. decipiens* for cod in the southwestern Gulf of St. Lawrence.

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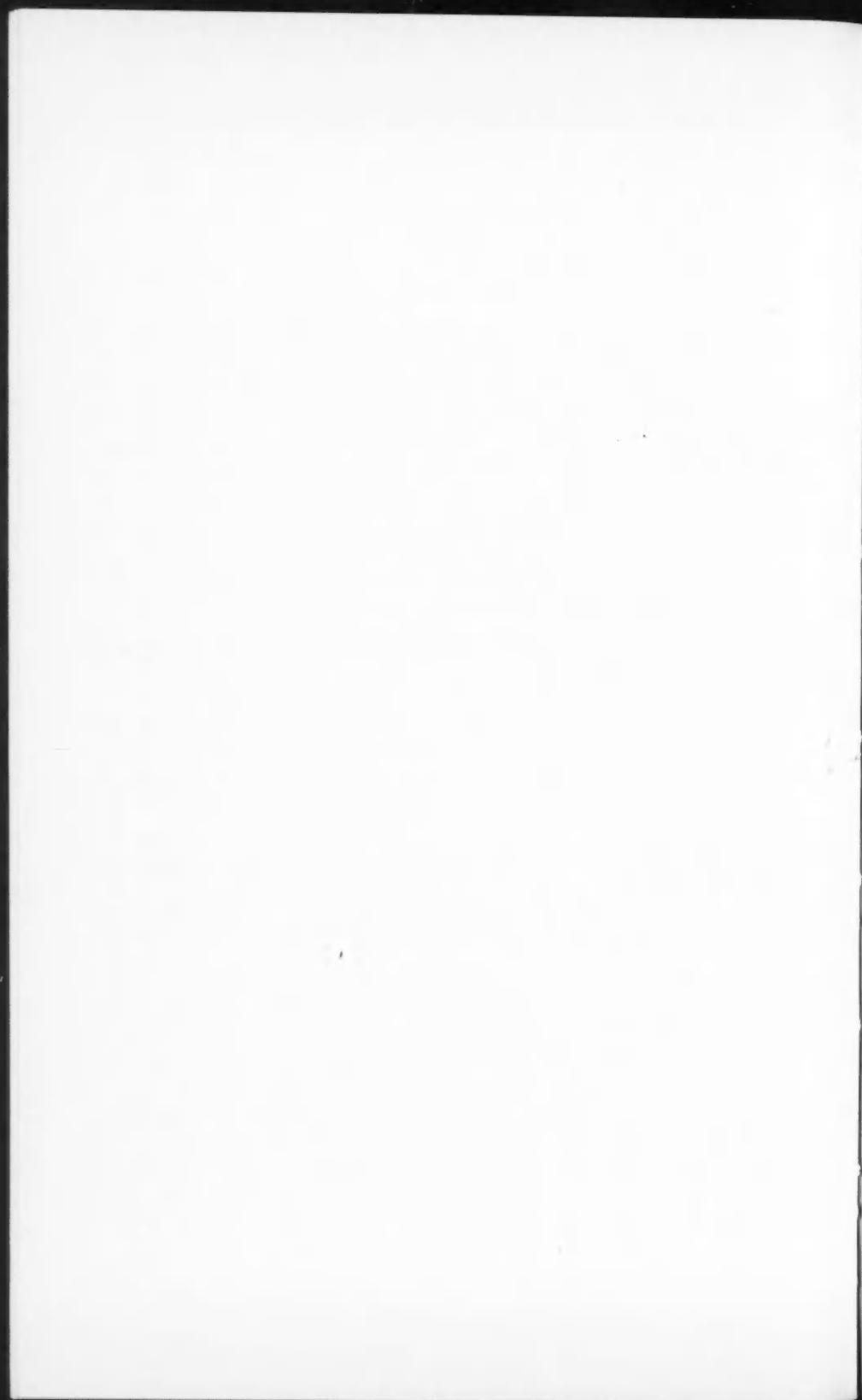
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Spoilage of Fish in the Vessels at Sea: 6. Variations in the Landed Quality of Trawler-caught Atlantic Cod and Haddock During a Period of 13 Months^{1, 2}

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ABSTRACT

A study has been made of the landed quality (*i.e.* the quality at the time of discharge from the vessels) of trawler-caught, gutted and iced market cod and large haddock. The rate of deterioration, as indicated by the rise in TMA values, varies with the time of the year. Poorer quality fish are landed during the colder months of November and December and also during the warmer summer months of June, July, and August. The best quality fish are landed during the months of February, March, April, May and September.

This seasonal spoilage pattern is similar for cod and for haddock and has been observed in the landed quality of the fish from eight individual trawlers over a period of 13 months.

Some of the probable factors that bring about this seasonal pattern in the spoilage rate of these fish are discussed.

INTRODUCTION

THE TERM "LANDED QUALITY" is used to indicate the state of preservation of the fish at the time it is discharged from the vessel at the plant.

Most east coast trawlers remain at sea for periods of not more than 10 days. At the time of landing the fish will have been iced in the holds from 2 to 9 days. Obviously there will be some difference between the landed quality of first- and last-caught fish. In order to compare the landed quality of different lots of fish it is necessary, therefore, always to take into consideration the number of days that the fish have been in stowage.

The work described in this paper has been done to determine whether the period of the year during which the fish are caught has any effect on their landed quality.

The test period extended over 13 months, from November 1956 to November 1957, inclusive. To begin with, there were only seven trawlers in the fleet, but after the investigation had been under way for 4 months another trawler was added. These vessels fished principally on the off-shore banks east of Nova Scotia and south of Newfoundland, including the Grand Bank, Green Bank, St. Pierre Bank, Quereau Bank, Western Bank and the waters around Sable Island.

EXPERIMENTAL METHODS

In order to minimize variations in spoilage rate arising through differences in the size of the fish, only large haddock (3 to 7 lb) and market cod (3 to 8 lb) were used. Special care was also taken not to use fish that had been stored directly against the wooden shelves and pen boards. Apart from these two specifications

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the fish to be used from each day's catch were picked indiscriminately from the pens at the time they were being discharged at the wharf.

For each species (cod and haddock), 10 fish were taken from each day's catch that had been in stowage for 3 to 9 days inclusive. These groups of 10 similar fish taken from each day's catch will be referred to as "lots". There were very many days when fish of the required size and species were not caught. One trip theoretically could provide 14 lots of fish to test, but very often there were less than half this number of lots.

The fish to be tested were placed in special containers and taken immediately into the cutting room of the plant where one fillet was removed from each fish. These fillets were then quick-frozen in a contact-type freezer, packed in boxes, and delivered by truck to this laboratory where they were held at approximately -12°C until tested. Preliminary tests with 50 fish showed that they could be held in frozen storage for 30 days under these conditions without significant loss in weight or change in trimethylamine (TMA) values. In most cases the storage period before testing was less than 1 week and none was over 30 days.

The degree of deterioration that had taken place in these gutted cod and haddock was determined by measuring the TMA content. The procedure used was that given by Dyer (1945, 1950) and presented in greater detail by Hoogland (1956). It has been shown previously that with specific exceptions, the TMA values for fillets freshly cut from cod and haddock are closely correlated with the

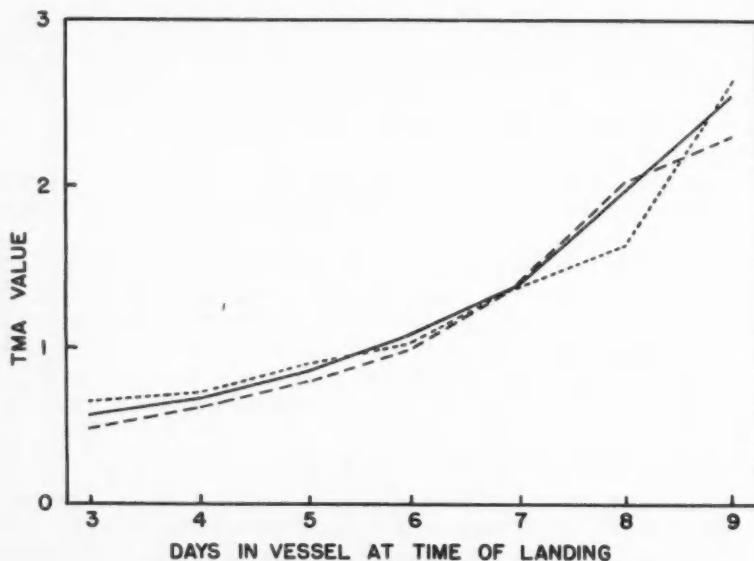


FIG. 1. Comparison of the mean TMA values from 9550 mixed cod and haddock (continuous line), 3860 haddock (broken line) and 3120 cod (dotted line) that had been from 3 to 9 days in the vessel. These values were all obtained from large haddock and market cod.

quality of the fish (Castell and Greenough, 1958; Hoogland, 1958). Those exceptions, when TMA is not always a measure of quality for cod and haddock, are confined chiefly to fish that have developed bilgy odours through contact with dirty pen boards and certain other types of spoilage that develop in ungutted or improperly gutted fish (Castell *et al.*, 1958). For this reason ungutted fish and those stored directly against wooden surfaces were not used in these tests.

EXPERIMENTAL RESULTS

COMPARISON OF TMA VALUES FOR COD AND HADDOCK

It was important to know whether TMA values have the same significance for the two species, cod and haddock, used in these tests. Previous observations (Castell and Triggs, 1955) suggested that the TMA values for cod were slightly higher than those for haddock for any given degree of deterioration.

Figure 1 gives the curves showing the increase in TMA with time for 3860 large haddock and 3120 market cod that had been held 3 to 9 days in the vessels. The same Figure also contains a similar curve for a mixed group of 9550 cod and haddock. Although during these periods of holding, the mean values for the cod were very slightly higher than those for the haddock, the difference was extremely small. For all practical purposes it may be assumed that deterioration of such cod

TABLE I. Number of fish tested, monthly mean TMA values and standard errors (S.E.) for 4- to 9-day fish over a 13-month period from November 1956 to November 1957, inclusive.

Period		4 days	5 days	6 days	7 days	8 days	9 days
Nov. 1956	No.	90	110	100	60	40	20
	Mean	0.79	1.19	1.16	2.43	3.57	3.50
	S.E.	0.03	0.07	0.08	0.24	0.17	0.47
Dec. 1956	No.	110	120	90	100	60	40
	Mean	0.79	1.18	1.57	1.58	4.47	4.96
	S.E.	0.05	0.10	0.10	0.11	0.38	0.45
Jan. 1957	No.	70	160	150	100	110	20
	Mean	0.51	0.68	0.84	1.32	2.24	2.82
	S.E.	0.05	0.06	0.05	0.07	0.14	0.31
Feb. 1957	No.	160	200	130	160	140	50
	Mean	0.53	0.71	0.76	1.32	1.10	1.74
	S.E.	0.02	0.03	0.03	0.06	0.04	0.17
Mar. 1957	No.	80	90	120	100	80	20
	Mean	0.48	0.51	0.94	1.11	1.42	2.46
	S.E.	0.02	0.02	0.06	0.07	0.11	0.46
Apr. 1957	No.	230	310	230	130	140	40
	Mean	0.55	0.60	0.70	1.11	1.48	1.99
	S.E.	0.02	0.02	0.02	0.06	0.09	0.13
May 1957	No.	270	220	240	180	180	60
	Mean	0.55	0.68	0.85	1.16	1.46	1.78
	S.E.	0.02	0.03	0.04	0.06	0.09	0.09
June 1957	No.	260	210	300	230	90	10
	Mean	0.74	0.92	1.28	1.42	1.85	1.82
	S.E.	0.02	0.02	0.04	0.04	0.10	0.02

TABLE I: *continued on next page.*

TABLE I: *continued*

Period		4 days	5 days	6 days	7 days	8 days	9 days
July 1957	No.	80	150				
	Mean	1.03	1.00	1.40*	1.80*	2.40*	3.10*
	S.E.	0.08	0.06				
Aug. 1957	No.	120	110	100	60	60	30
	Mean	0.90	1.10	0.87	2.22	2.31	3.60
	S.E.	0.06	0.07	0.04	0.20	0.19	0.39
Sept. 1957	No.	100	140	110	100	60	0
	Mean	0.45	0.72	0.89	1.03	1.11	-
	S.E.	0.02	0.06	0.06	0.08	0.08	-
Oct. 1957	No.	110	190	180	70	80	40
	Mean	0.57	0.71	0.94	1.11	1.82	2.54
	S.E.	0.05	0.06	0.08	0.06	0.11	0.21
Nov. 1957	No.	180	200	200	150	80	40
	Mean	0.77	0.89	1.31	1.52	3.02	2.32
	S.E.	0.05	0.04	0.06	0.07	0.22	0.24

*Approximations only.

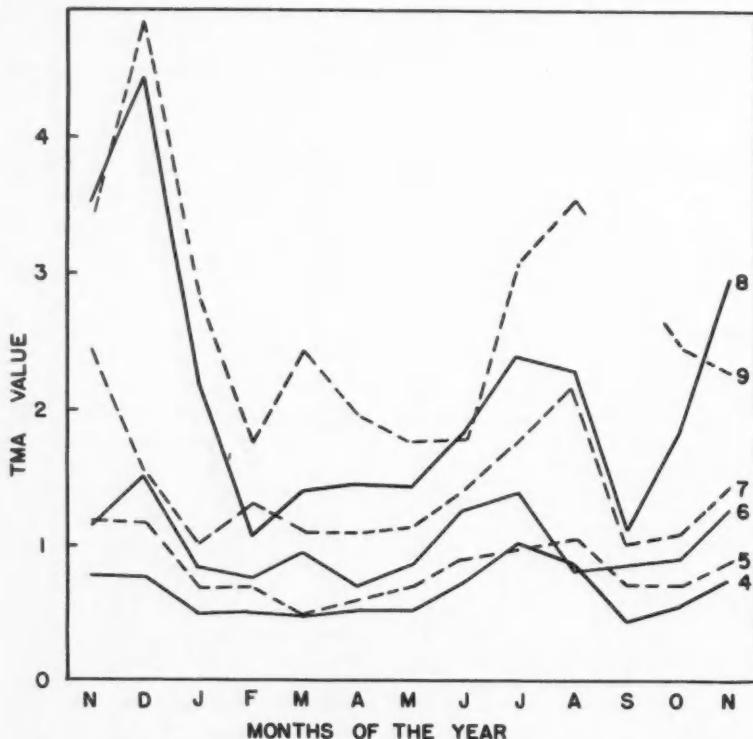


FIG. 2. Seasonal variations in the landed quality of 9550 mixed cod and haddock from the whole fleet as indicated by the monthly mean TMA values for the 4-, 5-, 6-, 7-, 8- and 9-day fish.

and haddock, as indicated by the accumulation of TMA in the muscle, takes place at the same rate under similar storage conditions.

SEASONAL CHANGES IN THE TMA VALUES

Table I gives the monthly mean TMA values, and their standard errors, for the 4- to 9-day mixed cod and haddock landed by all the trawlers during the 13-month test period. (Because of errors in making some of the tests during July the values for the 6- to 9-day fish for this month are approximations only). In Fig. 2 these results are given in the form of a graph which clearly shows a seasonal trend

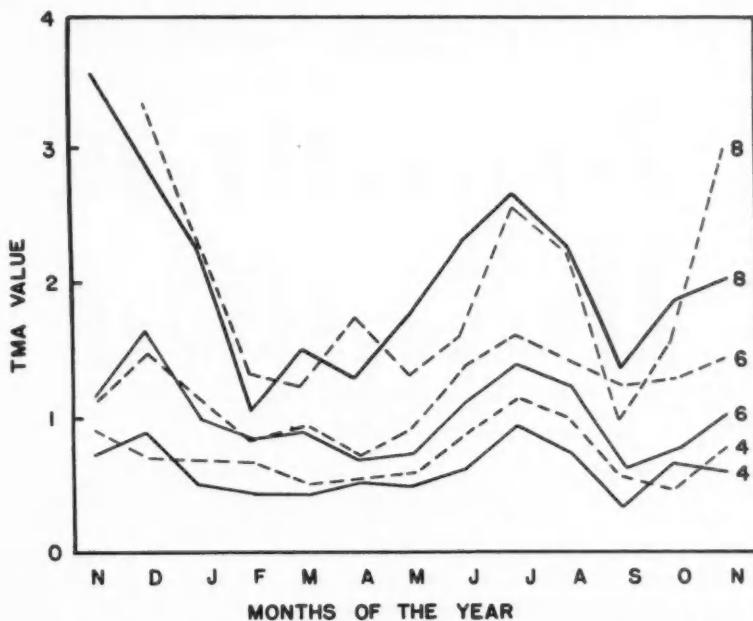


FIG. 3. Seasonal variation in the landed quality of 3120 cod (broken lines) and 3860 haddock (continuous lines) as indicated by the mean monthly TMA values for 4-, 6- and 8-day-old fish.

in variations of the TMA values. The characteristics of these curves, already showing in the 4-day fish, became more pronounced as the storage period lengthened. The TMA values were lower during the months of February, March, April and May and for another period during September and October. Higher values were obtained during November and December (1956), for the summer months of June, July and August and again for November (1957). Similar shaped curves are obtained when the values for 3680 haddock and 3120 cod are treated separately (Fig. 3).

The mean TMA values for the 4- to 8-day mixed cod and haddock during the whole 13-month test period were 0.65 and 1.90 respectively. Another picture

of the seasonal variation in quality can be obtained by determining the percentages of these fish each month that were above and below these mean values for the year (Fig. 4). November, December and the three summer months yielded the highest proportion of fish with TMA values above the mean for the year.

SEASONAL TMA VALUES FOR FISH FROM INDIVIDUAL TRAWLERS

In the results from the individual vessels we are necessarily confronted with far fewer lots of fish in each age group per month, than from the whole fleet. Also there were periods when each individual trawler was not fishing because of time

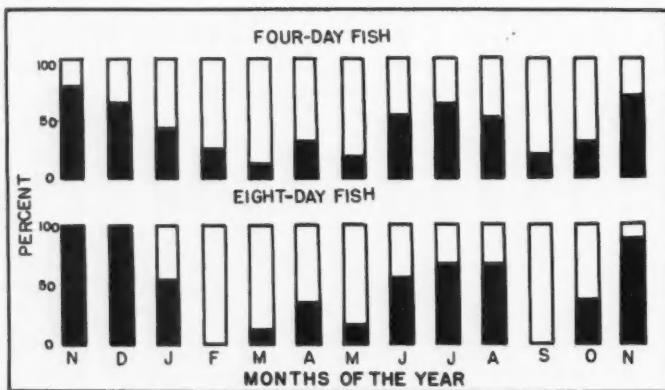


FIG. 4. The monthly percentages of 4- and 8-day combined cod and haddock that were above (in white) and below (in black) the mean TMA values for the 4- and 8-day fish for the 13-month test period. A similar pattern was also obtained for the 5-, 6- and 7-day fish. The mean TMA value for the 4-day fish was 0.65 and that of the 8-day fish was 1.90.

out for their annual refit or for other reasons. Nevertheless, where sufficient numbers of tests were available the data from four individual trawlers showed the same seasonal trend as was found for the whole fleet (Fig. 5).

It was observed that the similarity between the seasonal pattern of the individual trawlers and that of the whole fleet was greatest with those vessels that were known to take greatest care of their fish. This was particularly so with the older fish that were stored in the vessels for 7 and 8 days. In other words, when more care was taken of the fish, the characteristic seasonal pattern became more evident.

SEASONAL VARIATIONS IN THE GRADES OF FISH

For those who are interested in the commercial aspects of fish quality, the results of these tests can be interpreted to give a very close approximation to the percentages of the different grades of fish landed each month. This is based on the close correlation between the quality of fish and their TMA values, which showed that for gutted cod and haddock, values under 1.0 corresponded to Grade I fish and

values of 1.1 to 7.0 corresponded to Grade II fish (Castell *et al.*, 1958; Hoogland, 1958). Out of the 9550 fish there were less than 20 with TMA values over 7.0, and these were so close to 7.0 that they will be included here with the Grade II.

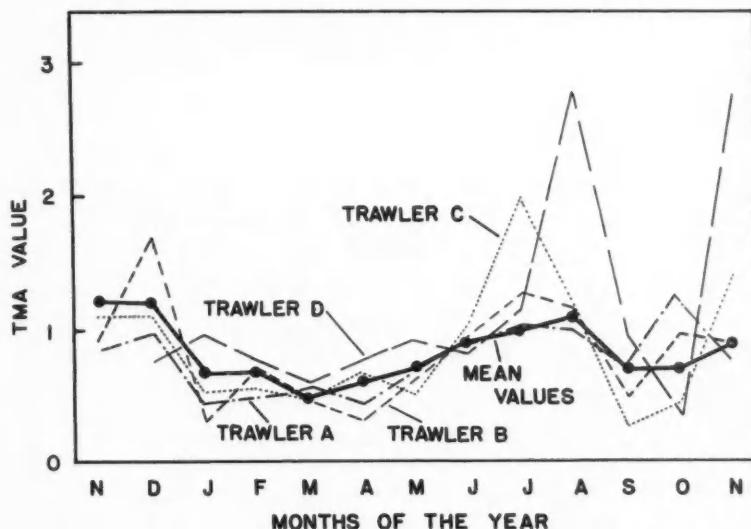


FIG. 5. Monthly mean TMA values of 5-day fish from four individual trawlers (A, B, C, D) compared with mean values of similar data for 5-day fish from the whole fleet of eight trawlers.

Figure 6 shows the monthly percentages of the 8-day fish that were Grade I and Grade II. Once again the characteristic seasonal trend is evident. Of the 8-day fish landed in February, March, April, May and September, 40 to 50% were Grade I quality while almost no Grade I fish of this age were landed in November

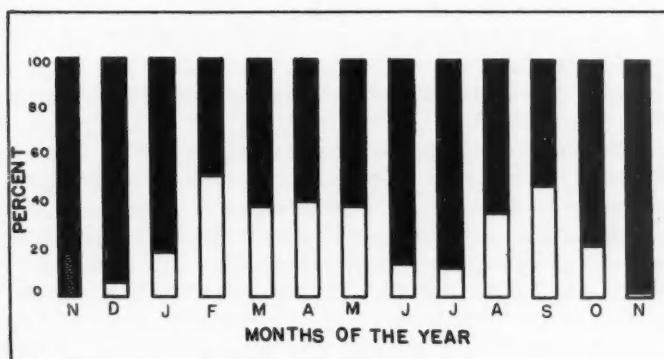


FIG. 6. The monthly percentages of Grade I (in white) and Grade II (in black) 8-day-old fish from the whole fleet.

and December, and very little more in January, June and July. The percentages of Grade I fish landed each month for each age category are given in Table II. The balance of the fish were Grade II.

DISCUSSION

From the results that have been recorded in this paper, there seems little doubt that the quality of the fish at the time of landing does show a pattern of variation throughout the year. Whether or not this seasonal spoilage pattern is repeated year after year can only be established by further observations.

TABLE II. Percentage of 4-, 5-, 6-, 7- and 8-day-old large gutted cod and haddock with Grade I quality that were landed during 13 monthly periods, from November 1956 to November 1957, inclusive. These percentages are based on tests of 8800 fish.

Month	Percent Grade I fish				
	4-day-old fish	5-day-old fish	6-day-old fish	7-day-old fish	8-day-old fish
November	77	43	58	16	0
December	77	58	32	29	5
January	90	85	73	35	18
February	96	84	78	41	51
March	100	97	63	59	38
April	94	86	80	47	40
May	92	85	76	51	39
June	81	61	37	29	14
July	70	74	32*	27*	12*
August	74	63	68	30	37
September	97	81	66	64	47
October	93	79	77	46	22
November	78	72	42	29	3

*Approximations only.

It should be emphasized that primarily this has been a study of the rate of deterioration of the fish in the vessels. It does not deal with the initial quality of the fish at the time they are taken from the water. It has indicated that the fish caught in November and December spoiled faster than similar fish caught in February or March. It has not shown that freshly caught fish in November or December are of quality inferior to that of freshly caught fish in February or March. It might well be that fish caught earlier in the winter are initially more acceptable, from the standpoint of flavour or texture, than those caught later in the winter or in early spring.

One of the most interesting problems raised by these observations is the nature of the relationship between the period of the year and the landed quality of the fish. Is it the result of some inherent difference in the fish at the time they are taken from the water? Or are the causal factors to be found in differences in the environment in which the fish are gutted and stored at different periods of the year?

Although only few data are available, it has generally been taken for granted that the spoilage rate of iced fish in trawlers is influenced by many different factors. These spoilage factors can be roughly grouped into those associated with the living fish before it has been caught, and those that act primarily on the spoilage organisms after the fish has been captured. Some factors, such as feeding habits, may

have a physiological effect on the living fish and also directly affect the post-mortem environment of the fish, e.g. by the presence or extent of faecal contamination on the exterior surfaces of the fish. Some of the more important of these factors that are believed or are known to affect spoilage are as follows:

1. Those that are associated with the living fish:

- A. Feeding habits; the type of food, and whether the fish is feeding or not feeding.
- B. The geographic location of the fishing grounds on which the fish are caught, which includes a complex of environmental factors.
- C. The sex and the changes in the habits and physiology of the fish associated with spawning.
- D. Specific differences in the chemistry of the fish.
- E. The method used for catching the fish, and in particular, the degree to which the fish have struggled immediately prior to death.

2. Those that are associated with dead fish and have their action primarily on the spoilage organisms:

- A. The spoilage microflora including bacteria from the sea water, the slime and the faeces of the fish, the ice, and from incidental contamination on the trawler.
- B. The care taken by the fishermen in handling, gutting, and storing the fish.
- C. The thoroughness with which the fish are chilled and iced.
- D. Temperature, including that of the fish at the time they are taken from the water, the atmospheric temperature and the degree to which the fish are exposed on the deck before being iced in the hold; the ambient temperature of the fish hold which helps control the rate at which the ice melts; and temperature during subsequent storage.

It will require a great deal more information than is now available before it can be decided which of these many factors are those that determine the seasonal changes in the landed quality of the fish that have been observed. It is also probable that as the seasons change, some of these factors may lose or gain in importance and that the spoilage rate is governed by a number of factors that are always in a state of flux.

During the last decade numerous papers have been published that deal with various phases of the biology of cod and haddock, their geographical distribution and the environment in which they live. At first sight one might expect that the perusal of these papers would throw some light on the observations that have been made in this work. As a typical example one might cite "Relation Between Feeding and the Sexual Cycle of the Haddock", by Homans and Vladykov (1954). These authors have collected data showing the monthly variation in the numbers of feeding haddock, the monthly variation in the amount of food taken by haddock and the monthly variation in the percentage of haddock in the different phases of the sexual and spawning cycle. It would appear to be a simple matter to compare

and correlate the seasonal variations in the landed quality of haddock with some of these seasonal cycles of feeding and spawning. It is interesting to note their statement that "during the spring months (February to May) less than 25% of all haddock examined had food in their stomachs, whereas for the remainder of the year as many as 82% of the haddock examined were feeding. It is also during the spring months that the least amount of food is eaten". Quite apart from the effect of the abstinence from feeding on the physiology of the fish (and particularly the activity of the gut enzymes), during this period there would be very much less faecal contamination of the fish during handling and gutting on the deck of the trawlers. This could very well be a partial explanation of the better keeping quality of the fish caught during this season. It would not explain the second period of better quality in the landed fish which occurs in September, when the number of feeding haddock is at a maximum.

Spawning of haddock, according to these authors, takes place between January and June and is almost completed by the end of May. This means that during the late spring and summer there may be large percentages of "spent" fish among the catches, which may in turn be at least partially responsible for the high percentages of soft-textured fish taken during this period.

However, one cannot carry these comparisons to too great a detail. The period for spawning varies from one geographical location to another and at any specific period in the spring and early summer there will be some fish in each of the ripening, spawning, spent and recovery stages. As the season progresses it is the proportion in each stage that changes. The same applies to the feeding cycle.

The fish used in the work described in this present paper on the landed quality were not differentiated into feeding and non-feeding fish; nor was any attempt made to classify them into stages of their sexual maturity or spawning cycle. If a correlation is to be looked for between these different stages in the biological conditions of the fish and their post-mortem spoilage rate, the details of the biological condition of each individual fish must first be ascertained.

The accumulated biological data that are now available are of immense interest to those concerned with the factors contributing to post-mortem spoilage. But their value lies chiefly in suggesting what some of these factors may be and in the development of future plans for their study.

This work on the landed quality of fish is being continued. As a result of the experience already gained, special attention will be paid to two aspects of the problem. First, an attempt will be made to determine whether the same seasonal spoilage pattern occurs year after year; second, an effort will be made to ascertain the feeding and sexual stages, and other pertinent biological data, of each individual fish that is to be tested.

ACKNOWLEDGMENT

This work has been facilitated through the generosity of the management of the Maritime National Fish Company of Halifax. They not only made it possible for us to utilize their plant facilities and their fleet of eight deep-sea trawlers, but they also provided us, without cost, with all the fish that were used in these tests.

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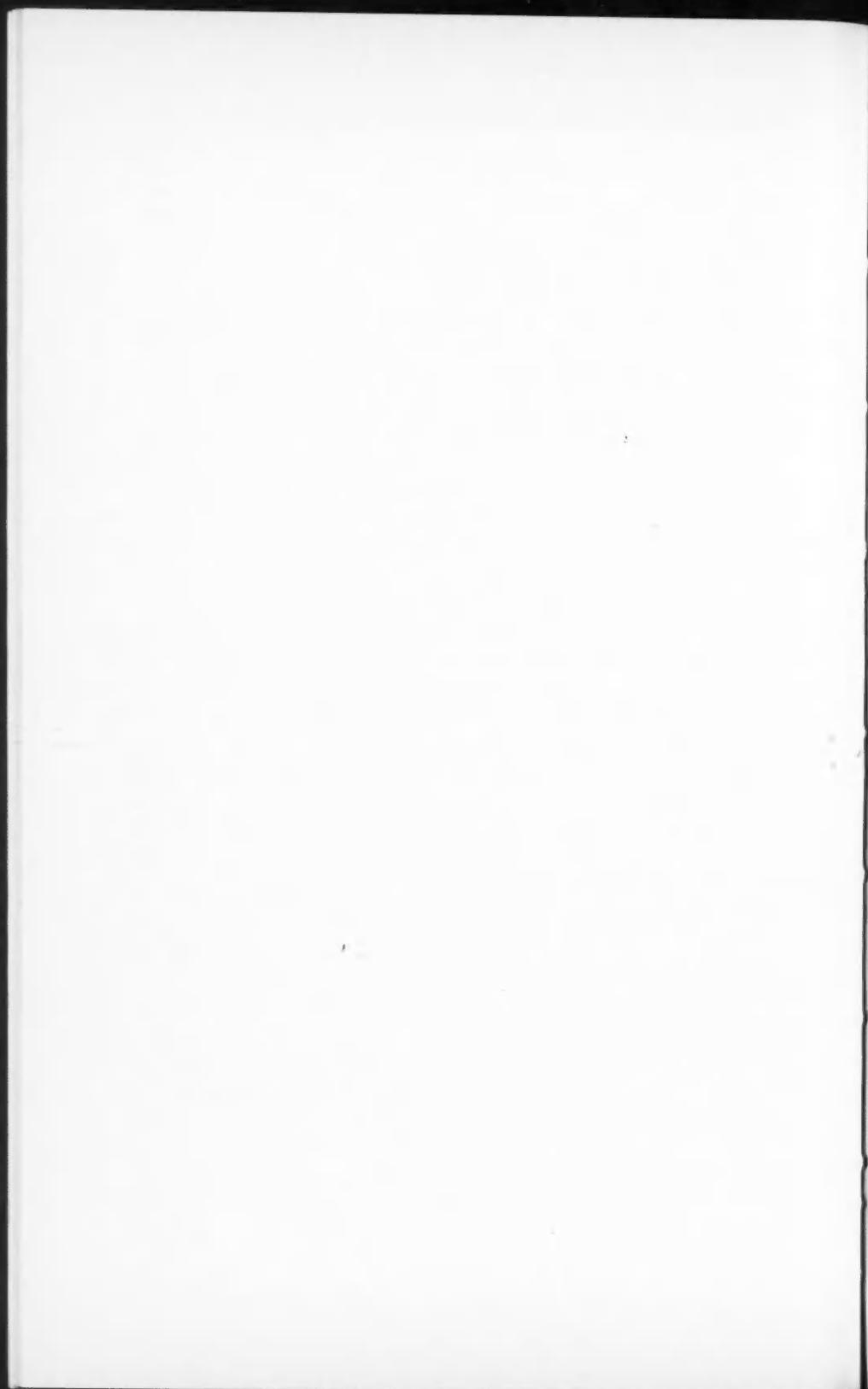
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Biochemical Studies on Sockeye Salmon During Spawning Migration. V. Cholesterol, Fat, Protein and Water in the Body of the Standard Fish^{1, 2}

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ABSTRACT

The absolute changes in fat, protein, water and free and esterified cholesterol for the entire body of standard male and female sockeye salmon (*Oncorhynchus nerka*) during the Fraser River spawning migration are discussed.

The data were obtained for twelve groups of males and twelve groups of females, totalling 216 fish. The fish were caught at three locations: before entering the river, 250 miles up the river, and at one of the spawning grounds 715 miles from the first sampling station.

INTRODUCTION

RECENTLY, a pure race of Fraser River sockeye salmon (*Oncorhynchus nerka*) was sampled before the fish entered the river (Lummi Island, Wash., U.S.A.), at Lillooet 403 km up river in British Columbia, and at the end of an 1135-km spawning migration (Forfar Creek, B.C.). The data showed that the large number of fish taken (216) and the uniformity of the population permitted an accurate designation of a standard sockeye for both sexes. In effect, the realization of a standard fish permits one to obtain the data which would be obtained if a fish could be analysed, then returned to the water and recaptured at a later time. By this technique not only percentage changes but absolute weight changes in tissue components may be determined.

The first report in this series described the weight and/or length changes which occurred in all major organs and tissues during spawning migration and in addition discussed the changes in plasma cholesterol and electrolyte levels (Idler and Tsuyuki, 1958). Subsequent reports discussed the changes in fat, protein, free and esterified cholesterol, nitrogen compounds and water in sockeye salmon flesh (Idler and Bitners, 1958; Duncan and Tarr, 1958; Wood, 1958).

The present report discusses the changes in fat, protein, water and free and esterified cholesterol in the entire eviscerated fish (entire body), *exclusive of the flesh*, and compares and contrasts the changes with the corresponding changes which occurred in the flesh of the standard fish of either sex. The viscera of Stuart Lake sockeye have been shown to contribute very little fat and protein, 2 to 4%, as compared with the body (Idler and Clemens, in press).

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²Part IV of this series appeared in *Can. J. Biochem. and Physiol.*, 36: 833-838 (1958).

METHODS

The sockeye salmon were caught in reef nets, anaesthetized in ice water, and bled by severing the caudal artery. The fish were placed in polyethylene bags, stored on "dry ice" and finally were partially thawed overnight and the frozen viscera, including the kidneys, were removed. The head and tail were combined with the interiorly well-scraped skin and scraped bones, and the flesh was kept separate as previously described (Idler and Tsuyuki, 1958). The frozen samples were divided into four groups for both sexes at each location and were then blended with a silent electric cutter. Representative 1-lb (454-g) samples were heat processed in enamelled cans and used for the analyses described in this report. The calorie (Cal) is the large or kilogram-calorie, and fat is taken as equivalent to 9.3 Cal/g and protein to 4.1 Cal/g. Fat, protein, water and cholesterol were determined as previously described (Idler and Tsuyuki, 1958; Idler and Bitners, 1958).

CHANGES IN PERCENTAGE COMPOSITION

The water content of the combined head, skin, bones and tail (hereinafter referred to as "combined trimmings") was much smaller than for the flesh for both sexes at Lummi Island and Lillooet but the difference was much smaller at the spawning grounds. The water content of the combined trimmings from both sexes increased even more on a percentage basis than did that of the flesh during the last 750 km of the migration (Table II).

The fat content of the combined trimmings from both sexes was twice as great as that of the flesh at Lummi Island. At Lillooet the combined trimmings had 3 times the fat content of an equal weight of flesh and this decreased to 1.5 times on arrival at the spawning grounds.

The protein content of the combined trimmings from both sexes remained essentially constant throughout the river migration. By contrast, the protein content of the flesh continued to decrease as the fish moved up the river. The ratio of the protein content of the remainder of the body to that of the flesh was similar for both sexes at each of the three locations, averaging 0.74 at Lummi Island, 0.78 at Lillooet and 0.94 at Forfar Creek.

The total cholesterol levels of the remainder of the body were more than double those of the flesh at all locations for both sexes. Similarly, the ratio of free

TABLE I. Mean values of weights of body of sockeye salmon during spawning migration*.

Site	Sex	No. of fish	Trimmings	Flesh
Lummi Island	♂	23	grams	grams
Lillooet	♂	40	881 ± 33 **	1467 ± 21
Forfar Creek	♂	25	871 ± 10	1302 ± 51
Lummi Island	♀	33	1057 ± 10	1304 ± 40
Lillooet	♀	40	785 ± 15	1335 ± 13
Forfar Creek	♀	55	765 ± 20	1135 ± 30
			834 ± 12	934 ± 10

* See Idler and Tsuyuki (1958) table II for weight of live fish, etc.

** Mean ± standard error.

TABLE II. Proximate analyses and cholesterol content of the body of migrating sockeye salmon.

Components	Sex	Location					
		Lummi Island		Lillooet		Forfar Creek	
		Trimmings	(Flesh)*	Trimmings	(Flesh)	Trimmings	(Flesh)
Moisture %	♂	60.2 ± 0.7**	(67.2)	64.5 ± 0.2	(70.7)	77.1 ± 0.4	(78.2)
	♀	59.4 ± 0.3	(67.0)	64.8 ± 0.5	(71.0)	76.3 ± 0.3	(78.0)
Fat %	♂	20.1 ± 0.8	(9.34)	15.9 ± 0.5	(5.07)	4.7 ± 0.3	(3.23)
	♀	21.7 ± 0.5	(10.55)	15.2 ± 0.2	(5.59)	4.3 ± 0.3	(2.70)
Protein N %	♂	2.57 ± .01	(3.51)	2.52 ± .01	(3.28)	2.55 ± .01	(2.68)
	♀	2.60 ± .02	(3.51)	2.64 ± .02	(3.38)	2.63 ± .02	(2.84)
Free cholesterol mg/100 g	♂	60.9 ± 3.5	(25.7)	65.6 ± 1.0	(30.9)	59.8 ± 1.0	(31.1)
	♀	63.9 ± 0.6	(25.6)	64.8 ± 0.5	(30.6)	58.3 ± 0.3	(34.7)
Total cholesterol mg/100 g	♂	71.2 ± 3.1	(29.2)	83.5 ± 2.8	(34.1)	78.2 ± 0.9	(36.7)
	♀	70.2 ± 0.6	(29.0)	79.7 ± 2.4	(32.6)	76.2 ± 2.2	(35.9)

*Flesh values included for comparative purposes. For further details see Idler and Tsuyuki, 1958; Idler and Bitners, 1958.

**Mean ± standard error.

cholesterol in the remainder of the body to that in the flesh exceeded 2 for both sexes at all locations except Forfar Creek, where the ratio was slightly less than 2.

It has previously been pointed out that the cholesterol content of the flesh of both sexes continually increased during the migration, even though the reserves of fat, protein and plasma cholesterol were depleted so extensively. It can now be seen that the entire body of the fish of both sexes appears to maintain cholesterol at a high level throughout the entire river migration.

CHANGES IN THE STANDARD FISH

WATER

The percentage composition data shown in Table II and the weights of the combined trimmings (and of the flesh) for the standard fish (Table I) were employed to calculate the changing composition of the standard fish (Table III).

The standard female lost 4% of its body water between Lummi Island and Lillooet and regained it between Lillooet and Forfar Creek (Table III). The flesh consistently lost water; 9.8% between Lummi Island and Lillooet and 9.6% between Lillooet and Forfar Creek. The remainder of the body gained 6.4% water between Lummi Island and Lillooet and 28% between Lillooet and Forfar Creek. The loss of water by the flesh is thus balanced by the water uptake of the remainder of the body resulting in no net loss of body water during the river migration.

The standard male lost 2% of its body water between Lummi Island and Lillooet and gained 24% between Lillooet and Forfar Creek for a net increase of 21%. The flesh lost 6.6% and gained 10.7% water between Lummi Island and Lillooet, and Lillooet and Forfar Creek, respectively, for a net increase of only 3.4%. The remainder of the body gained 6% water between Lummi Island and

TABLE III. Fat, protein and cholesterol in the body of migrating sockeye of standard length.

Flesh component	Sex	Location					
		Lummi Island		Lillooet		Forfar Creek	
		Trimmings	Flesh	Trimmings	Flesh	Trimmings	Flesh
Moisture	♂	530	(986)	562	(921)	815	(1020)
	♀	466	(894)	496	(806)	636	(729)
Fat	♂	177	(137)	138	(66)	50	(42)
	♀	170	(141)	116	(64)	36	(25)
Protein	♂	143	(322)	138	(267)	168	(219)
	♀	128	(294)	126	(240)	138	(165)
Free cholesterol	♂	0.537	(0.377)	0.571	(0.402)	0.632	(0.406)
	♀	0.502	(0.342)	0.496	(0.347)	0.486	(0.324)
Total cholesterol	♂	0.627	(0.428)	0.727	(0.444)	0.826	(0.479)
	♀	0.551	(0.387)	0.609	(0.370)	0.636	(0.335)
Ratio of free to total cholesterol	♂	0.86	(0.88)	0.79	(0.91)	0.76	(0.85)
	♀	0.91	(0.88)	0.81	(0.94)	0.76	(0.97)

Lillooet and 45% between Lillooet and Forfar Creek for a net increase of 54%. It can thus be seen that the flesh is secondary to the remainder of the body in contributing to the increased water content of the body of the standard male during the river portion of the spawning migration. The flesh gains only 34 g of water as compared to 285 g by the remainder of the body.

CHOLESTEROL

The standard male showed a steady increase in both the free and total cholesterol of the trimmings as the fish moved up the river (Table III). The standard female showed a steady increase in total cholesterol of the trimmings as the fish moved up the river but the free cholesterol levels decreased slightly.

The standard female showed a constantly decreasing ratio of free to total cholesterol in the combined trimmings as the fish moved up the river (Table III). By contrast the ratio of free to total cholesterol in the flesh showed a steady increase at the same locations. The standard male also showed a constantly decreasing ratio of free to total cholesterol in the trimmings as the fish moved up the river; however, the ratio of free to total cholesterol in the flesh of the male reached a maximum at Lillooet.

The changes in flesh cholesterol (Table II) of the standard fish have been discussed in a previous report (Idler and Bitners, 1958).

COMPARISON OF THE FLESH TO THE REMAINDER OF THE BODY AS A SOURCE OF FAT

The flesh was the major source of fat for both sexes from Lummi Island to Lillooet (Table IV). The standard female used 41% more fat from the flesh than from the rest of the body while the male used 81% more from the flesh. The source of fat reversed from Lillooet to Forfar Creek. The female used 102% more fat from the remainder of the body as compared to the flesh while the male used 266%

TABLE IV. Changes in fat and protein in the body of migrating sockeye salmon of standard length.

Site	Sex	Change in weight			
		Fat		Protein	
		Flesh	Trimmings	Flesh	Trimmings
<i>milligrams per kilometre of migration</i>					
Lummi Island to Lillooet	♂	-176	- 97	-137	-12
Lillooet to Forfar Creek	♀	-189	-134	-134	- 5
Lummi Island to Forfar Creek	♂	- 32	-117	- 65	+40
Lummi Island to Forfar Creek	♀	- 53	-107	-101	+16
Lummi Island to Forfar Creek	♂	- 82	-111	- 89	+22
Lummi Island to Forfar Creek	♀	-100	-116	-112	+ 9

more from the remainder of the body. Over the entire migration route from Lummi Island to Forfar Creek the standard female drew 16% more fat from the remainder of the body as compared to the flesh while the male drew 35% more.

COMPARISON OF THE FLESH TO THE REMAINDER OF THE BODY AS A SOURCE OF PROTEIN

The flesh was the major source of protein for both sexes in both segments of the river (Table IV). From Lummi Island to Lillooet the standard fish of both sexes used a small amount of protein from the remainder of the body. From Lillooet to Forfar Creek the portions of the body exclusive of the flesh of both sexes gained in total protein. The overall result from Lummi Island to Forfar Creek was a gain in protein of the remainder of the body for the standard fish of both sexes and the increase was three times as great for males as for females.

COMPARISON OF FLESH FAT AND PROTEIN CONSUMPTION

The standard female withdrew 41% more fat than protein from the flesh between Lummi Island and Lillooet while the male withdrew 28% more fat (Table IV). The consumption of flesh fat as compared to flesh protein reversed between Lillooet and Forfar Creek; the standard female withdrew 91% more flesh protein than fat and the male withdrew 103% more flesh protein. The overall effect between Lummi Island and Forfar Creek was that on a weight basis, 8.5% and 12% more protein than fat was withdrawn from the flesh of the standard male and female, respectively.

RELATIVE FAT, PROTEIN AND TOTAL ENERGY EXPENDITURES FROM THE ENTIRE BODY OF BOTH SEXES PER STANDARD FISH

The standard female expended 132% and the standard male 83% more fat than protein from the entire body between Lummi Island and Lillooet. From Lillooet to Forfar Creek the female expended 88% more fat than protein while the male expended 495% more fat.

The standard female expended 18.1% more fat and 6.7% less protein from the entire body than did the male over the 403-km migration from Lummi Island to Lillooet. This resulted in an expenditure of 13.5% more energy from the body of the female as compared to the male.

The standard female expended 6.6% more fat and 247% more protein from the entire body than did the male over the 749-km migration from Lillooet to the spawning grounds. This resulted in an expenditure of 23% more energy from the body of the female as compared to male.

During the entire migration from Lummi Island to Forfar Creek the female expended 11.4% more fat and 53.7% more protein from the entire body than did the male. This resulted in an expenditure of 17.4% more energy from the body of the female as compared to the male.

RELATIVE FAT, PROTEIN AND TOTAL ENERGY EXPENDITURES FROM THE ENTIRE BODY OF BOTH SEXES PER UNIT WEIGHT OF LIVE FISH

The preceding discussion does not take into account the differences in the live weight of the standard fish of the two sexes. The average live weight of the standard female was 2266 g and of the male 2468 g, from Lummi Island to Lillooet (Idler and Tsuyuki, 1958). The energy consumed from the body of the standard female was thus 1.57 Cal/kg/km as compared to 1.28 Cal/kg/km for the male or 23% more for the female than for the male.

From Lillooet to the spawning grounds the average live weight of the female was 2162 g and of the male 2477 g (Idler and Tsuyuki, 1958). The energy consumed from the body of the standard female was 0.85 Cal/kg/km as compared to 0.60 Cal/kg/km for the male or 42% more for the female than for the male.

In order to calculate the energy change from Lummi Island to Forfar Creek in terms of energy consumption per unit of live weight, it is necessary to consider the time the fish spent in the two phases of the migration. Lummi Island to Lillooet represents 10 days of travel at an average weight for males of 2468 g and 2266 g for females. Lillooet to Forfar Creek is 17 days and the average male weight 2477 g as compared to 2162 g for the female (Idler and Tsuyuki, 1958). Thus the best average weight of the standard male during the migration was 2475 g while that of the female was 2200 g. The energy consumed from the body of the standard female over the entire migration was 1.1 Cal/kg/km as compared to 0.84 Cal/kg/km for the male or 32% more for the female than for the male.

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NOTE

Proteins in Fish Muscle. 15. Note on the Preparation of Actin from Cod Muscle with Potassium Iodide¹

A convenient procedure for the preparation of actin from rabbit muscle and also from extracted rabbit actomyosin was described by Szent-Györgyi (1951a). It was based upon the observation that actin could be reversibly depolymerized by potassium iodide in the presence of a small concentration of adenosinetriphosphate (ATP) (Szent-Györgyi, 1951b).

This note reports the application of the procedure to actomyosin from cod muscle. The properties of the product indicated that it was fairly pure actin.

All preparative operations were carried out in a cold room (2 to 3°C), and all solutions were pre-cooled to this temperature. Fifty grams of muscle from post-rigor cod (*Gadus callarias*) was extracted twice in a Waring blender with 400 ml of K-phosphate buffer, ionic concentration ($\Gamma/2$) = 0.05, pH 6.6, to remove the albumins. The residue was then blended for one minute with 400 ml of 0.6 M KCl and K-phosphate, total $\Gamma/2$ = 0.8, pH 7.3. This extract was diluted with three volumes of water containing ATP and ascorbic acid (each 10^{-4} M), and the resulting precipitate dissolved in 6 M KI solution containing ATP and ascorbic acid (each 2×10^{-3} M) to give a final KI concentration of 0.6 M. After centrifuging to remove some insoluble material, the supernate was treated with one quarter its volume of 95% ethanol cooled to -40°C to denature the myosin. After centrifuging at $1500 \times g$, the supernate, containing some unsedimented precipitate, was dialyzed against frequent changes of water containing 10^{-4} M ATP and ascorbic acid, at pH 7.8 to 8.5. After centrifuging to remove the remaining insoluble protein, the yield was found to be 1.08 g of protein per 100 g of fish muscle.

Precipitation of the product with ammonium sulphate (0.9 M, pH 7.0) apparently caused extensive denaturation, so this procedure could not be used to increase the concentration of the extract. To achieve this, the protein was precipitated by adjusting the pH to 4.5 with acetate buffer (Tsao and Bailey, 1953). Precipitation was 90.5% complete, and the product redissolved completely in 5% NaHCO₃. The resulting solution exhibited strong birefringence, a characteristic property of F-actin.

Part of this solution was dialyzed against KCl-phosphate, $\Gamma/2$ = 0.4, pH 7.4, presumably yielding a solution containing F-actin, because of its strong birefringence. Another part of the solution in NaHCO₃ was dialyzed against water to convert the protein irreversibly to G-actin, but some birefringence persisted. The conversion was completed by dialysis against KI-phosphate, ($\Gamma/2$ = 0.8, pH 7.4, no ATP or ascorbic acid), and then against KCl-phosphate ($\Gamma/2$ = 0.4, pH 7.4). The resulting solution exhibited no birefringence, and presumably contained G-actin.

¹Part 14 of this series appeared in this JOURNAL, 16(1): 53-62, 1959.

The two states of the protein differed in heat stability, for when samples of the two solutions were heated at 100°C for 15 minutes, the protein in the "F-actin" solution precipitated almost completely, while the "G-actin" solution remained clear.

The electrophoretic pattern of the "G-actin" in 0.3 M KCl + K-phosphate, total $\Gamma/2 = 0.4$, pH 7.5, is shown in Fig. 1. The mobilities ($\times 10^5$ cm 2 volt $^{-1}$ sec $^{-1}$) of the main and minor peaks respectively were -4.58 and -6.12 (ascending)

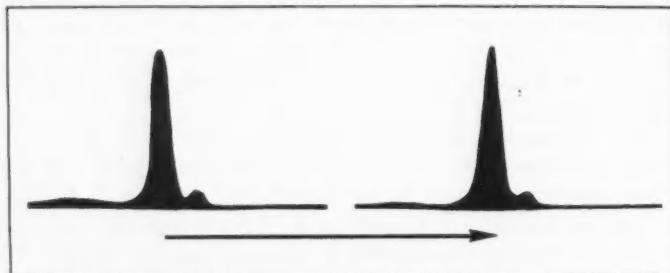


FIG. 1. Electrophoretic patterns of G-actin of cod.

Solvent, 0.3 M KCl + K-phosphate, total $\Gamma/2 = 0.4$, pH 7.53.
Field strength, 2.20 volts/cm. Time of electrophoresis, 333 min.
Temperature, 1.4°C. Pattern at left, ascending; at right, descending.

and -4.49 and -6.08 (descending). The mobility of the main peak agreed with that of the minor peak in an unfractionated KI-extract of cod muscle (Dingle, 1958), and also with the mobility of a G-actin prepared from cod according to a modification of the procedure of Tsao and Bailey (1953) by Odense and Dyer (unpublished). Connell (1954) has reported a mobility of -3.8×10^{-5} cm 2 volt $^{-1}$ sec $^{-1}$ under similar electrophoretic conditions for a G-actin prepared from cod muscle dehydrated with acetone. The mobility of the rapid peak in the present preparation appeared to be rather high for tropomyosin (Dingle and Odense, 1959), and may possibly have represented a polymer of actin.

The sedimentation patterns of the F-actin and G-actin preparations are shown in Fig. 2. Peaks 1 and 2 were not found in the G-actin solution. The observed sedimentation constants are given in Table I. The constant of peak 4 was close to that of cod tropomyosin (Dingle and Odense, 1959), which could be expected to occur as an impurity in this preparation, although electrophoresis did not definitely establish its presence. Peak 3 probably was due to G-actin; Connell

TABLE I. Sedimentation constants of cod actins*.

Peak No.:	1	2	3	4
F-actin	59.6	28.0	4.3	2.3
G-actin	4.6	2.1

*Given in Svedberg units, for the conditions of the run.

(1958) reported a constant of about 3 S for a cod actin prepared from acetone-dried muscle and ultracentrifugally purified. The presence of some of the peak 3 material in the F-actin preparation presumably meant that some of the actin was "inactive", or unable to polymerize.

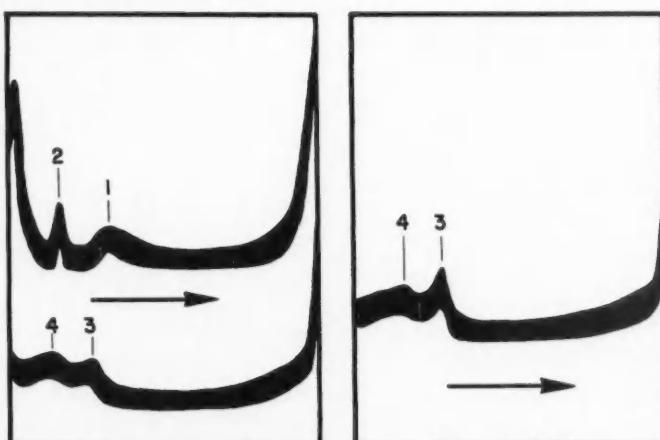


FIG. 2. Sedimentation patterns of cod actins.

Solvent, 0.3 M KCl + K-phosphate, total $\Gamma/2 = 0.4$, pH 7.4.
 Temperature, 18.4°C. Protein concentrations approximately 1 mg N/ml.
 Speed of centrifuging 59,780 rpm. Left, F-actin after 4 min (top) and
 after 52 min (bottom). Right, G-actin, after 52 min.

The KI procedure for the preparation of actin therefore appears to have been successful when applied to cod muscle actomyosin, and this confirms the previous evidence that this salt can dissociate cod actomyosin into myosin and actin (Dingle, 1958). A more detailed study of the product is proceeding.

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